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Biochemical impacts of some organophosphorus insecticides on cabbage plants

Biochemische Einwirkungen einiger Organophosphor-Insektizide auf Kohlpflanzen

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Summary

The side-effect of certain organophosphorus insecticides on some biochemical aspects of cabbage plants was studied. At growth period of 2 months, the field-grown plants were treated with the pesticides malathion, pirimiphos-methyl and prothiofos. The investigated insecticides were used at the recommended doses and sprays were repeated three times at intervals of 1 month. After each spray, randomized samples were taken after 0, 1 and 2 weeks of application and biochemically analyzed. The obtained results could be summarized as follows:

Chlorophyll analysis showed no significant variation after the first application. While continuous reduction was recorded after the second and third treatment.

Sugars and carbohydrate contents were depleted in the treated cabbage leaves after the consequent application with the studied pesticidal chemicals. Malathion induced the maximum decline followed by prothiofos and pirimiphos-methyl.

Free amino acids exhibited different responses as far as the individual impact of the used chemicals was considered. Total proteins content showed an increase after the first application in response to both malathion and prothiofos. However, all the used pesticides decreased protein contents of the treated cabbage leaves after the second and third application.

Elemental analysis revealed that N was declined after the second and third spray in particular as a function of the sprayed materials. On the other hand, P content was enhanced in the treated cabbage leaves in response to all the studied pesticides. K recorded no fixed trend after the different application with respect to the influence of the individual chemicals.

Key words: Malathion; pirimiphos-methyl; prothiofos; cabbage; chlorophyll; sugars; free amino acids; proteins; NPK

Zusammenfassung

Die Nebenwirkungen bestimmter Organophosphor-Insektizide auf einige biochemische Inhaltsstoffe von Kohlpflanzen wurden untersucht. Nach einer Wachstumsperiode von 2 Monaten wurden die Freilandpflanzen mit den Insektiziden Malathion, Pirimiphos-methyl und Prothiofos in den empfohlenen Aufwandmengen dreimal in Intervallen von je 1 Monat behandelt. Nach jeder Spritzung wurden randomisierte Proben 0, 1 und 2 Wochen nach Applikation entnommen und biochemisch untersucht. Die Ergebnisse können wie folgt zusammengefaßt werden:

Die Chlorophyllanalyse zeigte nach der ersten Applikation keine signifikanten Veränderungen, während betrachtet wurden. Der Gesamtproteingehalt nahm nach der ersten Applikation von Malathion und Prothiofos zu. Alle eingesetzten Insektizide verminderten jedoch den Proteingehalt in den behandelten Kohlblättern nach der zweiten und dritten Applikation.

Die Analyse der Elemente ließ eine Abnahme von N nach der zweiten und dritten Anwendung erkennen, besonders in Abhängigkeit von den gespritzten Verbindungen. Andererseits erhöhte sich der P-Gehalt in den behandelten Kohlblättern als Reaktion auf alle drei Insektizidanwendungen. Bei Kalium war kein fester Trend nach Applikation der einzelnen Insektizide erkennbar.

Stichwörter: Malathion; Pirimiphos-methyl; Prothiofos; Kohl; Chlorophyll; Zucker; freie Aminosäuren; Protein; NPK

1 Introduction

Current trends in population dictate intensified agricultural practices with concomitant growing use of agrochemicals, particularly in developing countries. Increased use of pesticides has greatly aided crops productivity, decreased losses of stored grains and has generally improved man's welfare. Because of their purpose, pesticides are intentionally toxic. Ideally, these chemicals should be poisonous only to target pest organisms; however, such as ideal is almost never fully attained. Thus, we must accept and dread with the unavoidable circumstance that the introduction of pesticides into the environment always carries with it some risk of unforeseen toxic consequences to non-target species.

In some cases, the utilization of pesticides (especially as foliar application) was found to be correlated with the appearance of some toxic symptoms and hazardous effects on plant growth. These impacts range from nearly inhibiting growth to gross morphological aberrations for the entire plant or only altered particular organs. Pesticides have been shown to alter cell division, cell enlargement and tissue differentiation as well as cause cellular and tissue deterioration. Consequently, these changes usually reveal growth inhibitors, epinasty, formative effects, albinism and reduced cuticle formation (Van Andel et al. (1976), Ashton and Crafts (1981), Anbudurai et al. (1986). On the other hand, leaf chlorosis followed by necrosis is a common response of plants treated with photosynthesis-inhibiting pesticides. Ultrastructural examination of these leaves usually reveals abnormal and degenerating chloroplasts as well as deteriorating cellular membranes as mentioned by Morrod (1976), Adhya et al. (1981) and Moorthy et al. (1992). Naturally, such impacts should be attributed to some physiological and biochemical disturbances in response to the pesticides application. That would urgently call for further studies regarding the effect of pesticide residues on plant metabolism. Surely, such studies will be valuable to recognize the problem of the side-effect of the pesticides on plant. This work was aimed to study the biochemical aspects of the insecticides malathion, pirimiphos-methyl and prothiofos applied to cabbage plants when used at the recommended doses.

2 Materials and methods

2.1 Insecticides

Malathion: S-[1,2-bis-(ethoxycarbonyl)-ethyl]-0,0-dimethyl phosphorodithioate (E. C. formulation Carbofos, 57 % a.i., at 1 liter/feddan).

Pirimiphos-methyl: 0-[2-(diethylamino)-6-methyl-pyrimidin-4-yl]-0,0-dimethyl phosphorothioate (E. C. formulation Actellic 50 % a.i., at 1.2 liter/feddan).

Prothiofos: 0-2,4-dichlorophenyl-0-ethyl-S-propyl-phosphorodithioate (E. C. formulation Tokuthion 50 % a.i., at 1.5 liter/feddan).

2.2 Cabbage plants

Cabbage seedlings were transplanted in field plots of 3 × 3 m area. After a growth period of 2 months, the experimental plots (containing around 25 plants) were treated with the individual insecticides as mentioned above. For each pesticide, three plots were distributed in random in addition to the unsprayed control plots. Spraying was repeated three times at intervals of one month.

After each spray, randomized plant samples were taken 0, 1 and 2 weeks after of the application pesticides. The outer leaves were used to study the biochemical changes as affected by the applied pesticides.

2.3 Chemical analysis

Chlorophyll content of cabbage leaves as determined in the ethanolic extract using the method of WINTERMANS and MOTS (1965).

Total carbohydrates were hydrolyzed using 0,5 mol/l H_2SO_4 in sealed tubes at 100 °C for 24 h. Total soluble sugars were extracted by 80 % ethanol for 6 h. Total carbohydrates and sugars were determined using the method described by DUBOIS et al. (1956). reducing sugars were determined in the ethanolic extract using the A. O. A. C. method (1985).

Total free amino acids were estimated in the ethanolic extract using the method of DOT et al. (1981). Total N and crude protein were estimated using the usual micro-Kjeldahl method (A. O. A. C. 1985).

P was determined according to CHAPMAN and PRATT (1961). K was determined in the digestion extract using Flame Photometer.

3 Results and Discussion

3.1 Chlorophyll contents

Data presented in Table 1 demonstrate the effect of the studied organophosphorus insecticides on chlorophyll content under the different consequent applications. Generally, there was no significant differences with regard to the used pesticides on chlorophyll "a", chlorophyll "b" and consequently

Table 1. Effect of some organophosphorus pesticides on chlorophyll content (mg/g d.wt.) of cabbage leaves
Tab. 1. Wirkung einiger Organophosphor-Insektizide auf den Chlorophyllgehalt (mg/g Tr.S.) in Kohlblättern

Pesticide	First spray				Second spray				Third spray			
	Weeks after application											
	Zero	One	Two	Mean	Zero	One	Two	Mean	Zero	One	Two	Mean
	Chlorophyll "a"											
Control	8.20	6.52	5.61	6.78	6.47	5.92	5.65	6.01	4.69	4.22	4.42	4.44
Malathion	8.20	6.78	6.89	7.29	5.58	4.97	4.62	4.89	3.92	3.66	3.18	3.59
Prothiofos	8.20	7.04	7.50	7.58	5.02	4.26	4.32	4.53	3.97	3.85	3.66	3.83
Pirimiphos-methyl	8.20	5.48	7.27	6.98	5.67	5.32	4.45	5.15	3.99	3.76	3.46	3.74
	Chlorophyll "b"											
Control	2.94	2.18	1.66	2.26	2.71	2.36	2.05	2.37	1.86	1.71	2.11	1.89
Malathion	2.94	2.87	2.06	2.62	2.34	1.75	2.21	2.10	1,39	1.42	1.27	1.36
Prothiofos	2.94	2.69	2.36	2.66	2.39	1.56	2.04	2.00	1.26	1.34	1.31	1.30
Pirimiphos-methyl	2.94	1.89	2.29	2.37	1.52	2.30	1.65	1.82	1.51	1.62	1.36	1.50
	Total Chlorophyll											
Control	11.14	8.70	7.27	9.04	9.18	8.28	7.70	8.38	6.55	5.93	6.53	6.33
Malathion	11.14	9.65	8.95	9.91	7.92	6.22	6.83	6.99	5.31	5.08	4.45	4.95
Prothiofos	11.14	9.73	9.86	10.24	7.41	5.82	6.36	6.59	5.23	5.19	4.97	5.13

changes included slight increases especially with plants treated with prothiofos and malathion.

Results of the second spray clearly demonstrate that there was noticeable decline in chlorophyll content as far as the different pesticides were concerned. In this consideration, the declines in chlorophyll "a" and total chlorophyll were more pronounced for plants treated with prothiofos rather than those treated with malathion or pirimiphos-methyl as compared with the untreated plants. On the other side, data of chlorophyll "b" revealed that pirimiphos-methyl produced the lowest values as compared with the other pesticides. In general, the magnitude of reduction in total chlorophyll content was 16.6, 22.1 and 16.8 % in plants sprayed with malathion, prothiofos and pirimiphos-methyl, respectively, as compared with the corresponding controls.

The third application of the used pesticides on cabbage plants resulted in further degradation in its chlorophyll content. However, the studied pesticides showed different magnitudes in relation to their capacity to reduce the pigment contents in plant leaves. The lowest values in chlorophyll "a" were recorded in plants treated with malathion followed by those treated with pirimiphos-methyl and prothiofos. On the other hand, readings of chlorophyll "b" showed that prothiofos induced the maximum reduction rather than malathion or pirimiphos-methyl. However, the values of total chlorophyll content showed the following trend: malathion < prothiofos < pirimiphos-methyl. In this regard, total chlorophyll content was found to be 78.2, 81.1 and 82,8 % as that of control with respect to plants treated with malathion, prothiofos and pirimiphos-methyl, respectively.

In most cases, however, the chlorophyll content of samples harvested 2 weeks later after the pesticides application was lower than those of 1 week sampling date.

Previous investigations revealed that the effect of organophosphorus insecticides on chlorophyll is variable, this is acceptable in view to the structural variability of these compounds and the plants under study. Blagonrauova and Kholchenko (1973) observed that parathion-methyl or fenitrothion increased leaf chlorophyll content of apples, while dichlorophon exhibited less effect. Hassan et al. (1978) reported reductions in chlorophyll of cotton plant leaves under stress of some organophosphorus insecticides. Afifi and El-Ballal (1982), reported that Sumithion and Dursban caused most significant reductions in chlorophyll compared with dimethoate, Gardona and Tamaron on three varieties of broad beans. Fais (1988), concluded that prothiofos increased significantly foliar chlorophyll in Navel oranges, while the same toxicant and malathion failed to induce any significant effect in Baladi oranges. Thus, it may be inferred that chlorophyll content may be affected either my increase or decrease as a result of organophosphorus compound application. The effect is dependent on the toxicant applied, the plant used and the growth stage which may be of significance and deserves some detailed studies.

3.2 Sugars and carbohydrates content

Generally, reducing sugars were depleted in cabbage plant leaves when treated with the different insecticides. However, this reduction was dependant on the individual pesticide and the sampling date under the different frequencies of application. Malathion exhibited the lowest values in reducing sugars followed by pirimiphos-methyl and prothiofos after the first and second application. However, the maximum decline in reducing sugars content of cabbage plant after the third spray was exerted by prothiofos followed by malathion and pirimiphos-methyl. The magnitude of decline as a function of application with malathion (for example) was 16.1, 28.1 and 24.8 % with respect to the first, second and third application, respectively.

More or less similar trends could be observed concerning the total soluble sugars content as influenced by the tested pesticides (Table 2). In this consideration, all the applied insecticides induced depletion in total soluble sugars content, however, the magnitude of this decline was greatly dependent on the individual toxicant and the frequency of application. Malathion exhibited the most pronounced reduction rather than prothiofos or pirimiphos-methyl. In this connection, the decline percent in total soluble sugars after the third application was 24.3, 8.1 and 5.8 % for malathion, prothiofos and pirimiphos-methyl, respectively, as compared with the unsprayed control. On the other hand, no regular trend in the depletion of total soluble sugars could be observed as far as the frequency of pesticide application was concerned. For example, the magnitude of reduction as influenced by prothiofos was 7.6, 6.0 and 8.1 % after the first, second and third spray, respectively. Such fluctuation may be attributed to plant growth stages which affect the physiological status and in turn affect the plant response for any external factor.

Data of total carbohydrates content showed similar results as those noticed for reducing and total sugars content (Table 2). Treating cabbage plants with the investigated organophosphorus insecticides induced a depressant effect on total carbohydrates content. Again, malathion was the most effective chemical in the reduction of total carbohydrates content as it exerted a decline of 13.2, 29.7 and 15.6 % with regard to the first, second and third spray, respectively. On the other side, either pirimiphos-methyl or prothiofos exhibited less impressive effect on total carbohydrates content as compared with malathion. Generally, the diversity showed between the individual insecticides with concern to their impact on sugars and carbohydrates content is expected and might be due to the mode of action and the capability of such agrochemicals to affect plant metabolism.

The obtained decline in sugars and carbohydrates content as induced by the applied pesticides might be attributed to a disturbance in carbohydrate synthesis due to the toxic impact of such pollutant on the enzymatic system which affect the different metabolic processes. This point of view would deserve further detailed investigations. The above mentioned results coincide with the findings of SHAWKY and SHAABAN (1974) that malathion decreased total sugars content in fruits of Baladi mandarins. GOMAA et al. (1976) indicated that dimethoate lowered total carbohydrates and its fractions when sprayed on peas. In addition, FAIS (1988), mentioned that prothiofos and malathion significantly reduced total carbohydrates in fruits of Navel and Baladi oranges. On the other hand, EL-REFAI et al. (1980), reported that Dipterex, prothiofos, Volaton and Metasystox did not significantly affect starch content in sugar beet leaves.

3.3 Free amino acids and total proteins

Free amino acids showed varied responses concerning the impact of the individual organophosphorus insecticides under investigation on cabbage leaves (Table 3). While, no apparent impact could be de-

Table 2. Effect of some organophosphorus pesticides on sugar and carbohydrate contents (mg/g. d.wt.) of cabbage leaves

Tab. 2. Wirkung einiger Organophosphor-Insektizide auf den Zucker- und Kohlenhydratgehalt (mg/g Tr.S.) von Kohlblättern

Pesticide	First spray				Second spray				Third spray			
	Weeks after application											
	Zero	One	Two	Mean	Zero	One	Two	Mean	Zero	One	Two	Mean
	Reducing sugars											
Control	7.17	7.17	7.30	7.20	8.04	7.83	8.30	8.07	7.77	7.90	7.07	7.58
Malathion	7.17	5.73	5.23	6.04	6.50	5.73	5.03	5.80	5.77	5.13	6.07	5.70
Prothiofos	7.17	6.23	6.10	6.50	8.47	8.07	5.90	7.50	5.87	5.70	6.37	5.60
Pirimiphos-methyl	7.17	6.07	5.97	6.40	8.30	8.03	5.10	7.10	8.93	7.17	6.50	7.50
	Total soluble sugars											
Control	42.30	40.30	42.70	41.80	41.70	43.00	40.00	41.60	44.00	42.30	39.70	42.00
Malathion	42.30	34.00	32.70	36.30	33.00	34.00	36.30	34.40	31.30	30.30	33.70	31.80
Prothiofos	42.30	35.70	35.30	37.80	37.30	39.00	41.00	39.10	39.70	38.30	37.70	38.60
Pirimiphos-methyl	42.30	38.30	32.70	37.80	39.00	41.00	40.60	40.20	43.70	40.30	35.30	39.80
	Total Carbohydrates											
Control	105.2	105.7	106.7	105.9	118.3	115.0	122.5	118.6	114.2	116.3	111.3	113.9
Malathion	105.2	91.7	78.7	91.9	86.7	84.2	79.2	83.4	103.3	95.0	90.0	96.1
Prothiofos	105.2	98.3	91.1	98.2	115.0	109.0	96.3	106.8	114.0	105.3	85.3	100.9

of cabbage plant

Tab. 3. Wirkung einiger Organophosphor-Insektizide auf den freien Aminosäuregehalt und das Gesamtproteingehalt (mg/g Tr.S.) von Kohlblättern

Pesticide	First spray				Second spray				Third spray			
	Weeks after application											
	Zero	One	Two	Mean	Zero	One	Two	Mean	Zero	One	Two	Mean
	Free amino acids											
Control	1.87	1.81	1.75	1.81	1.94	1.88	1.79	1.87	1.78	1.83	1.95	1.85
Malathion	1.87	1.79	1.77	1.81	2.03	1.75	1.88	1.89	1.98	1.86	1.69	1.84
Prothiofos	1.87	1.85	1.65	1.79	1.96	1.73	1.81	1.83	1.73	1.56	1.63	1.64
Pirimiphos-methyl	1.87	1.62	1.59	1.69	1.75	1.77	1.62	1.71	1.71	1.69	1.48	1.83
	Total proteins											
Control	140.6	159.4	157.5	152.5	170.6	168.1	163.1	167.5	151.5	145.0	165.0	153.1
Malathion	140.6	161.9	165.0	155.6	161.3	134.4	140.6	145.6	141.9	145.6	147.5	145.0
Prothiofos	140.6	175.0	161.9	159.4	164.4	142.5	146.3	151.3	148.8	151.9	142.5	147.5
Pirimiphos-methyl	140.6	153.8	161.3	151.9	163.1	153.1	158.1	158.1	159.4	150.6	139.4	149.4

tected in the mean values exerted by malathion, an observable decline was caused by pirimiphos-methyl during the consequent application. On the other hand, prothiofos exhibited slight reduction in free amino acids content after the first and second spray, however, a clear decrease occurred after the third application. Thus, no fixed pattern could be concluded regarding the impact of the used pesticides on free amino acids of cabbage plant leaves. In this connection, Afifi and El-Ballal (1982), reported that methyl parathion quantitatively reduced free amino acids content of wheat grains. Similarly, Fais (1988) mentioned that prothiofos and malathion depressed the total amino acids content of Baladi and Navel orange fruits.

Results of total proteins content showed that the frequency of the application was the limiting factor of the impact of the studied insecticides on cabbage plant leaves. Samples collected after the first application exhibited that both malathion and prothiofos induced an increase in the mean values of total proteins content of the treated plants, while pirimiphos-methyl showed a slight decline regarding this criterion. On the other side, plant samples taken after the second and third spray showed that all the applied pesticides had a depressant impact on total proteins content of cabbage plants. For both the second and third application, the capacity of the individual pesticides to reduce total proteins content could be arranged in following order: malathion > prothiofos > pirimiphos-methyl. Previous studies revealed that organophosphorus insecticides had varied impact on proteins content of the treated plants. Rozek and Marecrek (1981), reported that dimethoate and malathion exerted an increase in leaf proteins content of pea plants after 24 h of application, while a decline was observed in the later periods. Gomaa et al. (1976) concluded that dimethoate decreased total proteins content of pea plants. On other side, Dipterex, Tokuthion, Volaton and Metasystox had no significant impact on total proteins content of sugar beet as demonstrated by El-Refai et al. (1980).

3.4 Mineral content

Data demonstrated in Table 4 show the elemental content of cabbage plant leaves as affected by the consequent application of the organophosphorus insecticides.

N content showed limited variation in response to the sprayed pesticides after the first application. Only a slight increase was obtained as a function of the insecticide prothiofos. Taking the second and third application into consideration, it could be deduced that all the studied pesticides caused varied

Table 4. Effect of some organophosphorus pesticides on NPK content (mg/g d.wt.) of cabbage leaves
Tab. 4. Wirkung einiger Organophosphor-Insektizide auf den NPK-Gehalt (mg/g Tr.S.) von Kohlblättern

Pesticide	First spray				Second spray				Third spray			
	Weeks after application											
	Zero	One	Two	Mean	Zero	One	Two	Mean	Zero	One	Two	Mean
					Nitrogen							
Control	22.5	25.5	25.2	24.4	27.3	26.9	26.1	26.8	24.2	23.2	26.4	24.5
Malathion	22.5	25.9	26.4	24.9	25.8	21.5	22.5	23.3	22.7	23.3	23.6	23.2
Prothiofos	22.5	28.0	25.9	25.5	26.3	22.8	23.4	24.2	23.8	24.3	22.8	23.6
Pirimiphos-methyl	22.5	24.6	25.8	24.3	26.1	24.5	25.3	25.3	25.5	24.1	22.3	23.9
					Phosphorus							
Control	1.75	2.04	2.08	1.96	2.04	2.15	2.15	2.11	2.56	2.60	2.60	2.59
Malathion	1.75	2.23	2.73	2.30	2.20	2.33	2.60	2.38	2.96	3.04	3.06	3.02
Prothiofos	1.75	2.19	2.65	2.20	2.35	2.35	2.54	2.41	2.56	2.94	3.13	2.88
Pirimiphos-methyl	1.75	1.98	2.23	1.99	2.38	2.52	2.54	2.48	2.85	3.06	3.25	3.05
					Potassium							
Control	30.6	34.8	34.5	33.3	34.8	34.4	35.2	34.8	34.1	34.8	39.1	36.0
Malathion	30.6	32.6	35.6	32.9	37.6	39.2	39.6	38.8	38.9	37.3	34.3	36.8
Prothiofos	30.6	36.3	39.2	35.4	36.5	38.4	42.8	39.2	39.8	36.9	30.1	35.6
Pirimiphos-methyl	30.6	33.2	33.5	32.4	33.8	35.4	39.7	36.7	37.1	38.7	42.2	39.3

declines in N content of the sprayed cabbage leaves. In this connection, the lowest N concentration was detected in plants treated by malathion followed by those sprayed with prothiofos and pirimiphos-methyl. For instance, the reduction percent in mean values of N content after the second application was 13.1, 9.7 and 5.6 % with respect for malathion, prothiofos and pirimiphos-methyl.

P content showed different results rather than those observed for N in response to the sprayed pesticides. Generally, all the used organophosphorus insecticides caused an increase in P content of the treated cabbage leaves. That holds true for the different consequent applications. However, the volume of the induced increase was varied with the individual pesticides alongside the different experimental periods and applications. Malathion showed maximum P content after the first application, while pirimiphos-methyl exerted the highest P values after the second and third applications. The percent of increase as obtained after the third spray was 16.6, 11.2 and 17.8 % for malathion, prothiofos and pirimiphos-methyl, respectively.

K content exhibited different behaviour rather than that noticed for both N and P contents in responding to the investigated pesticidal chemicals. Data clearly revealed that there was no fixed trend regarding the impact of the individual insecticides on K content after the different frequent applications. In other words, some fluctuation (by decrease or increase) occurred during the different sampling dates and applications.

From the above mentioned data, it could be concluded that the determined elements had shown different phenomena with concern to its response to the applied pesticides. In this respect, KANSOUH et al. (1978) mentioned that both malathion and Sevin induced an enhancement in N and P content of the treated lettuce leaves. Similarly, HUSSEIN (1983), reported an increase in K content of soybean and cotton plants when sprayed with some organophosphorus insecticides. On the other side, FAIZ

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