THE INFLUENCE OF RIDOMIL PLUS 50% WP AND VYDATE 24%SL ALONE OR IN COMBINATION ON SOME BIOCHEMICAL CONSTITUENTS OF TOMATO LEAVES UNDER THE FIELD CONDITION

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Abstract

The influence of Ridomil plus 50% WP, Vydate 24% SL alone or in binary mixture on some chemical constituents were evaluated in tomato leaves at different intervals of application under the field conditions. The application of tomato seedlings with Vydate led to the highest significant decrease in chlorophyll (a, b & total) and carotene contents followed by the mixture treatment and the Ridomil plus alone in the case of chlorophyll (a) and total chlorophyll only. The recommended concentration of Ridomil plus resulted in higher chlorophyll (a) and carotene contents at different intervals of application comparing to the control treatment. On the contrary, Ridomil plus had a greater significant reducing in the total soluble sugar after 1, 7 and 14 days of application, while Vydate gave significant increase in the total soluble sugar after 1 and 7 days of application. The use of Ridomil plus, Vydate and their mixture caused a significant decrease in the total soluble protein after 14 days of application. However, the minimum decrease in the total soluble protein content was found in the treatment of Vydate. Generally, all treatments caused an increase in the total free amino acids until the first day of spraying, and then a decrease occurred until the end of experiment.

The nematicide, Vydate had no influence on the phenolic compounds of tomato leaves. On the contrary, Ridomil plus increased phenolic compounds in tomato leaves from 75.90, 77.45 and 78.51 in control plants to 88.74, 96.08 and 91.11 mg/g dry weight after 1, 7 and 14 days, respectively of application. Ridomil plus, Vydate and their mixture had a significant decrease in peroxidase activity of tomato leaves. Peroxidase enzyme can not restored its activity in treated tomato leaves with all treatments after 14 days of application.

Key words: Ridomil plus 50% WP, Vydate 24% SL, tomato leaves, chlorophyll, carotenes, soluble protein, soluble sugar, amino acids, phenols, peroxidase activity.

INTRODUCTION

Tomato is one of the most important vegetable crops in Egypt. Tomato plants are objected to several pests such as insects, nematodes, fungi, bacteria and viruses which may cause singly or in combination decreasing in yields by impeding plant

growth, retarding flowering, sucking juice and transmit viral diseases. Therefore, intensive use of pesticides is expected in order to grant high yield and quality of tomato fruits. Consequently, most of the work dealing with the efficiency of pesticides or their residues against pests (Saladin *et al.,* 2003)

Several investigators studied the effects of pesticides on biochemical compositions of vegetable plants. Abdel-All *et al.*, (1988) found that pesticides caused a significant increment in the amount of protein of soybean plants. El-Shahaat and Edrisha (1993) reported that chlorophyll concentration in cabbage leaves was increased by the insecticidal treatments. Ahmed and Siddiqui (1995) found that the fungicide Topsin–M significantly increased chlorophyll, protein and phenolic content of *Hibiscus esculentus* and *Capsicum annuum* (Pepper). El-Sherief and Salem (1998) mentioned that fungicides increased the contents of chlorophyll, nitrogen, ascorbic acid and soluble protein in potato leaves.

However, several studies have shown adverse effect of pesticides on chemical composition of plants. The herbicide Simazin had an impeding effect on the Hill reaction (Ashton *et al.,* 1960). Steward and Krikorion (1971) reported that carbonate derivatives of fungicide Antrakol, used for control of tobacco mildew, not only speed up cell division, but also inhibit photosynthesis. Stone *et al.*, (1987) found that the fungicide metalaxyl was absorbed, translocated, eventually accumulated in the lower leaves of *Brassica compestris* plants. Caseley *et al.*, (1996) noticed that pesticides inhibited the activity of some enzymes. Also, El-Sherif *et al.*, (1999) observed that insecticides were decreased the concentration of total carbohydrates in okra plants.

On the other hand, there are a little reports on the phytotoxicity of nematicides or fungicides and their mixtures with other products to vegetable plants. Applying a tank mix of pesticides can save time, labor, energy and equipment costs. Pesticides combination usually alters plant absorption and translocation as well as metabolism and toxicity at the side of action of one or more of the mixed products. Not all changes are for the better. Negative effects were occurring such as reduced pest control, increased damage to non-target (plants phytotoxicity and incompatibility problems between materials). Application of Ridomil plus 50% WP and Vydate 24% SL in tomato fields are very common but their physiological effects of each one or its mixture on the plants were unknown. Tomato plants are heavy infested with nematodes, early and late blight diseases, which causes considerable loss. Oxamyl is a systemic nematicide used for control of root knot disease on tomato, which cause by *Meloidogyne* spp. it absorbed by the foliage and roots, with translocation. The systemic fungicide metalaxyl is widely used in tomato production to control early and late blight diseases, which cause by *Alternaria solani* and *Phytophthora infestans*,

respectively. Therefore, the aim of the present study was to evaluate the effect of recommended rate of Ridomil plus 50% WP and Vydate 24% SL each alone or its mixture on various contents of tomato leaves, chlorophyll (a), (b) as well as (a+b), carotenoid, protein, total soluble sugars, free amino acids, phenolic compounds and the activity of peroxidase enzyme.

MATERIALS AND METHODS

I. Tested pesticides

1. Ridomil plus 50% WP: Metalaxyl (15%) + Copper oxychloride (35%Cu). The chemical name of metalaxyl is methyl N-(methoxyacetyl)-N-(2,6-xylyl) DL-alaninate. Ridomil plus 50% WP was recommended in Egypt against late blight disease on tomato at rate 150 g/100 L water.

2. Vydate 24% SL (Oxamyl). The chemical name of oxamyl is N, N-dimethyl-2-methyl carbamoyloxyimino-2-(methylthio) acetamide. Vydate 24% SL was recommended in Egypt against root knot disease on tomato at rate 3L/Fed. Both compounds were used at their recommended rates.

II. Determination of physico-chemical properties

The physico-chemical properties of spraying solutions of Ridomil plus 50% WP, Vaydate 24% SL and their mixture were determined. The pH values were estimated by Schott pH meter. Ostwold viscometer was used for measurement the viscosity. Also, foaming, electric conductivity, salinity percent and surface tension were determined.

III. Field experiment

Tomato plants *(Lycopersicon esculentim* Mill.) variety Super Strain B were planted in Bani-Sewif Governorate in winter season during 2006 year. The experimental area was divided according to the complete randomized blocks design including three replicates for each treatment. Each replicate was 6X7 m^2 (1/100 fed.). After 30 days of planting, tomato seedlings were sprayed as foliar treatment using a knapsack sprayer. The tested pesticides were used alone at their rates of application or in mixture at ratio 1:1 (v/v). The untreated control plots were sprayed with water. Common cultural and fertilization practices were followed. Homogenous tomato leaves were collected randomly after one hour, 1, 5, 7 and 14 days of application. The leaves were rinsed three times in distilled water, plotted on filter paper and divided into two sub samples, one was used fresh for the analysis of chlorophyll and carotene contents, whereas the other were dried at 70°C for 24 hrs. into a hot air drying oven and then ground into fine powder.

IV- Measurement of some biochemical constituents in tomato leaves after application

1. Determination of total chlorophyll and carotene contents

Chlorophyll and carotene contents were determined according to the method of Hiscox and Israelstam (1979). Chlorophyll contents were measured at 663 and 645 nm using Spectrophotometer and calculated by the equation of Arnon (1949), while the carotene contents were calculated by the equation of Villanueva *et al.* (1985).

2. Determination of the total soluble sugars

The extraction of total soluble sugars was prepared according to the method of Rosein (1957) and determined by phenol-sulfuric acid method as described by Dubois *et al.* (1956).

3. Determination of the total soluble proteins

The total soluble proteins were determined according to the method proposed by Lowry *et al.* (1951).

4. Determination of the total free amino acids

The method of Lee and Takahashi (1966) was followed for determination of total free amino acids.

5. Determination of phenolic compounds

Method of Ossipov *et al.* (2001) was used in extraction of total phenolic compounds from the powder of tomato leaves. The purified extract was used for the determination of phenolic compounds at 730 nm by Spectrophotometer according to the method of Torres *et al.* (1987).

6. Determination of peroxidase activity

The peroxidase activity was measured Spectrophotometrically according to the method of Boarkin (1972). One unit of enzyme activity defined as the amount of enzyme that cause change in optical density at 600 nm per minute at 25°C under standard assay condition. The specific activity of enzyme expressed as mOD units/min/mg protein. Proteins were measured with Folin reagent as described by Lowry *et al.* (1951) in the presence of bovine serum albumin as a standard.

V. Statistical analysis

The data were statistically analyzed using Costat Software Program (1985). Least Significant Differences test (LSD) was used for the comparison between treatment means at 5% level of probability according to Sendecor and Chachran, (1981).

RESULTS AND DISCUSSION

1- Physico-chemical properties of tested solutions

The physico-chemical properties, foaming, pH, salinity, surface tension and viscosity of Ridomil plus 50% WP, Vydate 24% SL and their mixture in tap, soft and hard water were studied (Table, 1). It was found that the Ridomil plus and Vydate were not caused foaming in the different types of water except Ridomil plus that gave foaming reach 4 cm^3 in hard water. In contrary, the mixture of both tested pesticides caused foaming reach 7 and 5 cm^3 in soft and hard water, respectively.

Mostly, the pH values of Ridomil plus, Vydate and their mixture in tap water were similar and ranged between 6.94 and 7.78. The spray solution of Vydate was acidic in soft and hard water, while it was alkali with Ridomil plus 50% WP and neutral for the mixture of both pesticides. The conductivity data in spraying solution of each pesticide and their mixture were higher in tap water than those in soft and hard water especially in case of Ridomil plus. The percentage of salinity in Ridomil plus solution was higher than that in Vydate solution but after mixing of them, it was decreased to 50% in all types of water.

Data indicated that the surface tension in Vydate solution was higher than that in Ridomil plus solution and their mixture. There were no differences in the viscosity of the solutions of Vydate, Ridomil plus and their mixture in tap, soft and hard water. The wetability of Ridomil plus in tap water was decreased sharply from 91.8 to 67.4 % after combination with Vydate. Slightly differences in wetability were found between Ridomil plus and their mixture with Vydate in soft and hard water.

II. Effect of tested pesticides on the chemical composition of tomato leaves

1. Effect on chlorophyll and carotene contents

Results recorded in Table (2) showed the effect of Ridomil plus 50 % WP, Vydate 24% SL and their mixture on the chlorophyll (a), chlorophyll (b), total chlorophyll and carotene contents of tomato leaves after one hour of spraying and also after 1, 5, 7 and 14 days of application. Data indicated that there were no significant differences between the treatments and control in their effect on chlorophyll (a) through all periods of evaluation except in the treatments of Vydate and its mixture with Ridomil plus at the end of experiment, which gave significant decrease as compared to control. The Ridomil plus treatment was slightly increased chlorophyll (a) concentration in tomato leaves from 0.700, 0.636, 0.683 and 0.671 mg/g fresh weight (f.w.) in control plants to 0.713, 0.676, 0.697 and 0.770 mg/g (f.w.) after 1, 5, 7 and 14 days of application, respectively. It was found that there were significant differences in chlorophyll (a) concentration of tomato leaves between the treatments of fungicide Ridomil plus and the nematicide Vydate within all evaluation periods except after 5 days of application.

Table 1. Physico-chemical properties of Ridomil plus 50% WP, Vydate 24% SL and their mixture in tap, soft and hard water.

Physico-chemical properties	Vydate 24% SL			Ridomil plus 50% WP			Mixture (Ridomil plus + Vydate)		
	Tap water	Soft water	Hard water	Tap water	Soft water	Hard water	Tap water	Soft water	Hard water
Foaming (cm^3)	–	–	–	–	–	4	Trace	7	5
pH	6.94	4.71	4.20	7.78	9.47	9.54	6.98	6.95	7.07
Conductivity	330	90	230	1000	380	210	350	130	150
Salinity (%)	0.20	0.10	0.20	0.40	0.20	0.20	0.20	0.10	0.10
Surface tension (dyne/cm)	72.00	68.86	72.00	44.00	42.81	40.86	38.63	46.59	42.00
Viscosity (m poise)	10.41	10.88	10.91	9.77	10.52	10.46	10.94	10.27	10.27
Wetability (%)	–	–	–	91.84	78.51	85.18	67.40	82.95	88.88

Generally, the three treatments, Ridomil plus, Vydate and their mixture decreased the chlorophyll (b) content in tomato leaves compared with the control treatment in all evaluation times. Data showed that the higher decrease in chlorophyll (b) content was obtained by Vydate, followed by its mixture with Ridomil plus and then Ridomil plus alone. It was found that chlorophyll (b) content was decreased significantly from 0.570, 0.824, 1.057, 1.240 and 0.957 mg/g f.w. in control treatment to 0.470, 0.671, 0.900, 0.965 and 0.625 mg/g f.w. in Vydate treatment after one hour of spraying and also after 1, 5, 7 and 14 days of application, respectively. The mixture of Ridomil plus and Vydate caused particularly significant decrease in chlorophyll (b) content of tomato leaves after 1 and 14 days post application as compared with the control treatment. Ridomil plus caused only significant decrease in chlorophyll (b) of tomato leaves at the end of experiment. Mostly, chlorophyll (b) was reduced more than chlorophyll (a) with all treatments especially Vydate treatment in all tested times.

The same trend of treatments effect on chlorophyll (b) was also found with contents of total chlorophyll, which decreased at the different intervals of application in all treatments. The high decrease in total chlorophyll content was obtained by Vydate, followed by its mixture with Ridomil plus and then Ridomil plus alone. Non significant differences in total chlorophyll were found between all the treatments and control after 7 days of application. In contrary, the reductions were significantly in all treatments comparing with the control after 14 days of application.

Data in the same Table (2) indicated that carotene contents in tomato leaves were decreased by the both treatments, Vydate and its mixture with Ridomil plus. Significant decrease in carotene contents was obtained after 1 h. and 1 & 14 days of application in the treatment of Vydate, while it was after 1 and 7 days of application in the mixture treatment. On the other hand, it was found that the application of Ridomil plus on tomato plants only led to increase of carotene contents in tomato leaves at different intervals of application which were 0.138, 0.198, 0.258, 0.301 and 0.232 mg/g f.w. comparing with 0.136, 0.197, 0.237, 0.275 and 0.183 mg/g f.w. in control treatment after 1 h. and 1, 5, 7 & 14 days of application, respectively. The significant increase in the carotene contents was found only in tomato leaves after 14 days of application with Ridomil plus.

Generally, results indicated that the treatments of Ridomil plus, Vydate and their mixture decreased chlorophyll and carotene contents of tomato leaves in comparison with control plants at different intervals of application. Also, It was observed that the spraying of tomato seedlings with Vydate led to the highest significant decrease in chlorophyll (a,b & total) and carotene contents followed by the mixture of Vydate with Ridomil plus and then Ridomil plus alone in case of chlorophyll

(b) and total chlorophyll only. Application of Ridomil plus gave mixed responses regarding quantities of chlorophyll and carotene contents of tomato leaves at different intervals of application. The recommended concentration resulted in higher chlorophyll (a) and carotene contents and lower chlorophyll (b) and total chlorophyll at different intervals of application comparing with the control treatment. Although there were no relation between the chlorophyll and carotene concentrations with the time elapsed, the highest decreasing rate of chlorophyll (b) and total chlorophyll was found in tomato leaves after 14 days of application with Ridomil plus, Vydate and their mixture. The highest increasing rate in chlorophyll (a) and carotene contents was also obtained after 14 days of spraying the tomato leaves with Ridomil plus.

Arteca, (1996) noted that the negative influence of pesticides on chlorophyll concentration may be due to the inhibition of the biosynthesis of aminolevulinc acid or protochlorophyllide reductase as a result of their inhibitory effect on the biosynthesis of gibberellins and cytokinins.

The present study indicated that the recommended concentration of Ridomil plus 50% WP resulted in higher chlorophyll (a) and carotene contents. These results agree with those obtained by El-Sherief and Salem (1998), who indicated that fungicides increase the total chlorophyll concentration. El-Sherief *et al.* (1999) mentioned that the increase in concentration of chlorophyll could ascribe to the good growth of plant foliage in pesticide-treated plots. Tort and Türkyilmaz (2003) suggested that the recommended rate of application for Captan 50% WP gave higher amount of chlorophyll (a) as well as (a+b) and carotenoid contents of pepper leaves than the higher concentration and control, while the amount of chlorophyll (b) and total chlorophyll was reduced in all the treated plots. Also, El-Ghinbihi and El-Sherief (2004) found that the fungicide Oxycarboxin was increased the concentration of total chlorophyll in cowpea plants.

On the other hand, The chlorophyll (a,b & a+b) and carotene contents in tomato leaves were clearly decreased by the both treatments, Vydate and its mixture with Ridomil plus. These results supported by Salem (1994) who found that the concentration of total chlorophyll in bean plants was reduced by using pesticides. Also, Selim and Attalla (2001) reported that pesticides sharply decreased the total chlorophyll in tomato plants. El-Sherief and El-Ghinbihi (2003) found that the acaricide Sanmite and the fungicide Topsin-M caused significant reduction in total chlorophyll concentration in tomato plants.

Table 2. Effect of Ridomil plus 50% WP, Vydate 24% SL and their mixture on chlorophyll and carotene contents of tomato leaves at different intervals of application.

Treatments	Chlorophyll (a) (mg/g fresh weight)	Chlorophyll (b) (mg/g fresh weight)	Total Chlorophyll (mg/g fresh weight)	Carotene contents (mg/g fresh weight)
After one hour of application				
Ridomil plus 50% WP	$0.768^{a} \pm 0.007$	$0.560^{a} \pm 0.010$	$1.290^{a} \pm 0.070$	$0.138^{a} \pm 0.006$
Vydate 24% SL	$0.650^{b} \pm 0.060$	$0.470^{b} \pm 0.010$	$1.150^{b} \pm 0.100$	$0.112^{b} \pm 0.003$
Ridomil plus 50% WP + Vydate 24% SL	$0.680^{b} \pm 0.040$	$0.550^{a} \pm 0.050$	$1.220^{ab} \pm 0.040$	$0.135^{a} \pm 0.010$
Control	$0.720^{ab} \pm 0.048$	$0.570^{a} \pm 0.020$	$1.330^{a} \pm 0.016$	$0.136^{a} \pm 0.003$
LSD	0.080	0.050	0.120	0.010
After 24 hours of application				
Ridomil plus 50% WP	$0.713^{a} \pm 0.021$	$0.819^{a} \pm 0.067$	$1.384^{b} \pm 0.036$	$0.198^{a} \pm 0.011$
Vydate 24% SL	$0.650^{b} \pm 0.006$	$0.671^{b} \pm 0.015$	$1.189^{a} \pm 0.050$	$0.158^{b} \pm 0.002$
Ridomil plus 50% WP + Vydate 24% SL	$0.649^{b} \pm 0.024$	$0.699^{b} \pm 0.006$	$1.325^{b} \pm 0.096$	$0.169^{b} \pm 0.001$
Control	$0.700^{ab} \pm 0.046$	$0.824^{a} \pm 0.035$	$1.535^{a} \pm 0.090$	$0.197^{a} \pm 0.006$
LSD	0.140	0.070	0.120	0.010
After 5 days of application				
Ridomil plus 50% WP	$0.676^{a} \pm 0.037$	$0.974^{ab} \pm 0.083$	$1.603^{ab} \pm 0.0125$	$0.258^{a} \pm 0.012$
Vydate 24% SL	$0.620^{a} \pm 0.032$	$0.900^{b} \pm 0.040$	$1.480^{b} \pm 0.031$	$0.220^{b} \pm 0.009$
Ridomil plus 50% WP + Vydate 24% SL	$0.500^{a} \pm 0.030$	$0.937^{ab} \pm 0.083$	$1.582^{b} \pm 0.027$	$0.226^{b} \pm 0.015$
Control	$0.636^{a} \pm 0.034$	$1.057^{a} \pm 0.045$	$1.728^{a} \pm 0.018$	$0.237^{ab} \pm 0.020$
LSD	0.200	0.130	0.130	0.030
After 7 days of application				
Ridomil plus 50% WP	$0.697^{a} \pm 0.010$	$1.112^{ab} \pm 0.70$	$1.795^{ab} \pm 0.091$	$0.301^{a} \pm 0.007$
Vydate 24% SL	$0.626^{b} \pm 0.037$	$0.965^{b} \pm 0.139$	$1.626^{b} \pm 0.175$	$0.236^{ab} \pm 0.033$
Ridomil plus 50% WP + Vydate 24% SL	$0.662^{ab} \pm 0.043$	$1.102^{ab} \pm 0.054$	$1.726^{ab} \pm 0.086$	$0.267^{b} \pm 0.012$
Control	$0.683^{ab} \pm 0.026$	$1.240^{a} \pm 0.029$	$1.935^{b} \pm 0.018$	$0.275^{a} \pm 0.018$
LSD	0.060	0.160	0.290	0.100
After 14 days of application				
Ridomil plus 50% WP	$0.770^{a} \pm 0.055$	$0.756^{b} \pm 0.026$	$1.419^{b} \pm 0.047$	$0.232^{a} \pm 0.016$
Vydate 24% SL	$0.574^{c} \pm 0.038$	$0.625^{c} \pm 0.083$	$1.274^{c} \pm 0.070$	$0.151^{c} \pm 0.021$
Ridomil plus 50% WP + Vydate 24% SL	$0.623^{bc} \pm 0.042$	$0.701^{bc} \pm 0.032$	$1.247^{c} \pm 0.069$	$0.170^{bc} \pm 0.008$
Control	$0.671^{a} \pm 0.013$	$0.957^{a} \pm 0.065$	$1.725^{a} \pm 0.041$	$0.183^{b} \pm 0.006$
LSD	0.070	0.110	0.110	0.030

2. Effect on the total soluble sugar

Changes were observed in total soluble sugar in tomato leaves at different intervals after the application with the tested pesticides (Table, 3). Application of different treatments gave mixed responses regarding quantities of total soluble sugar, which Ridomil plus had a greater significant reduction in the total soluble sugar after 1,7 and 14 days of application followed by its mixture with Vydate, which was after 7 and 14 days only. The total soluble sugar content was decreased significantly from 30.47, 26.87 and 25.10 in the control treatment to 28.80, 22.07 and 18.43 mg/g dry weight after 1,7 and 14 days of application by Ridomil plus, respectively. These results may be due to inhibition of nutritional cycles that responsible of the nutrient uptake and transport as a result of pesticides interference (Thomas, 1986: Nofal *et al.* 1988 and Nofal *et al* 1993). Also, Ismail (2000) and El-Sherief and El-Ghinbihi (2003) found that the pesticides that they tested decreased the total soluble sugar concentration in wheat grains and tomato fruits.

In contrary, the nematicide Vydate gave significant increase in the total soluble sugar after 1 and 7 days of application, which reached 36.37 and 29.00 mg/g dry weight, respectively. Results showed that there were significant differences between the three treatments, Ridomil plus, Vydate and the mixture of both during the experimental periods. The results obtained with Vydate treatment were complete agreement with those outlined by Ahmed (1993), Marei (2002) and El-Sherief and Abdallah (2002) who reported that the treatments, Propamocarb hydrochloride, Mikal, Mancozeb and Ridomil plus caused a remarkable increase of sugar content especially at higher doses, except Propamocarb hydrochloride, which caused an insignificant decrease of the sugar content of squash leaves.

Table 3. Effect of Ridomil plus 50% WP, Vydate 24% SL and their mixture on the total soluble sugar in tomato leaves at different intervals.

Treatments	Total soluble sugar (mg/g dry weight) at different intervals of application		
	1 day	7 days	14 days
Ridomil plus 50% WP	$28.80^{c} \pm 0.40$	$22.07^{d} \pm 0.26$	$18.43^{c} \pm 0.20$
Vydate 24% SL	$36.37^{a} \pm 0.37$	$29.00^{a} \pm 0.26$	$24.80^{a} \pm 0.65$
Ridomil plus 50% WP + Vydate 24% SL	$31.13^{b} \pm 0.66$	$24.30^{c} \pm 0.34$	$20.30^{b} \pm 0.52$
Control	$30.47^{b} \pm 0.32$	$26.87^{b} \pm 0.05$	$25.10^{a} \pm 0.05$
LSD at 0.05	0.89	0.48	0.82

3. Effect on total soluble protein

Results set up in Table (4) showed that Vydate had no or slightly effect on the soluble protein as compared with non-treated plants. However, Ridomil plus had a greater reducing effect on the total soluble protein during all experimental periods followed by its mixture with Vydate as compared to the control treatments. The Ridomil plus treatment significantly decreased the total soluble protein in tomato leaves from 38.33, 37.96, 31.53 and 27.40 mg/g dry weight in control plants to 33.20, 30.40, 24.47 and 18.46 mg/g dry weight after 1 h. & 1, 7 and 14 days of application, respectively.

The analysis revealed that the total soluble protein content was significantly decreased by spraying of tomato plants with the mixture of both tested pesticides, which reached 34.27, 27.27 and 22.47 comparing with 37.96, 31.53 and 27.40 mg/g dry weight in control treatment after 1, 7 and 14 days of spraying, respectively. The use of Ridomil plus, Vydate and their mixture caused a significant decrease in the total soluble protein after 14 days of application. However, the minimum decrease in the total soluble protein content was found in the treatment of Vydate. The present results were agreed with those of Singh and Mersie (2003) whom reported that the commercial formulation of metalaxyl contained various isomers and that these isomers may vary in phytotoxic effects on citrus leaves. They found that the R^- and S^+ isomers of metalaxyl differ in their biological activity and the isomer S^+ is responsible for the inhibition of protein synthesis. This finding show that the phytotoxicity of metalaxyl was due to the presence of the S^+ optical isomer and removal of this isomer from metalaxyl has enabled the continued use of this fungicide for control of foot rot and root rot diseases in citrus.

To clarify the above mentioned results, it could be attributed to an osmotic shock effect of systemic fungicides results in the release of protein and loss of membrane transport ability in the leaf cells (Amar and Rinhold, 1973). It has been suggested that the toxicant produced by the application of systemic fungicides inhibits protein synthesis by binding to the large ribosomal subunits inducing change in the enzyme system (Person *et al.*, 1957), ceasing ATP and NADP formation (Mishra and Waywood, 1968 and Siddiqui, 1997). Also, Siddiqui and Ahmed (2002) found that the systemic fungicides caused a significant decrease in total soluble protein of *Triticum aestivum* L. On the other hand, Tort and Tuekyilmaz (2003) reported that the fungicide Captan 50% WP had no influence on the protein contents of pepper leaves, as there was no significant difference in the treated and control treatments.

Table 4. Effect of Ridomil plus 50% WP, Vydate 24% SL and their mixture on the total soluble protein in tomato leaves at different intervals.

Treatments	Total soluble protein (mg/g dry weight) at different intervals of application			
	1 hour	1 day	7 days	14 days
Ridomil plus 50% WP	$33.20^{b} \pm 0.43$	$30.40^{c} \pm 0.34$	$24.47^{c} \pm 0.35$	$18.46^{d} \pm 0.26$
Vydate 24% SL	$38.70^{a} \pm 1.09$	$37.73^{a} \pm 0.20$	$31.53^{a} \pm 0.35$	$26.30^{b} \pm 0.20$
Ridomil plus 50% WP + Vydate 24% SL	$38.37^{a} \pm 0.23$	$34.27^{b} \pm 0.20$	$27.27^{b} \pm 0.15$	$22.47^{c} \pm 0.40$
Control	$38.33^{a} \pm 1.27$	$37.96^{a} \pm 1.27$	$31.53^{a} \pm 0.80$	$27.40^{a} \pm 0.91$
LSD at 0.05	1.30	1.23	0.95	0.99

4. Effect on total free amino acids

Results recorded in Table (5) showed the effect of both pesticides and their mixture on total free amino acids of tomato leaves. Data indicated that total free amino acids were not affected by all treatments except Vydate, which caused great significant increase of total free amino acids content of tomato leaves after 1h. and 1 day of application. It is clear that all treatments had a harmful effect on total free amino acids of tomato leaves after 7 days of application, which the highest significant reduction was found by Ridomil plus (19.7) followed by both treatments of Vydate and their mixture (23.7) comparing with control plants (28.5 mg/g dry weight). At the end of experiment, the total free amino acids content was severely inhibited in response to all treatments especially Vydate treatment, which caused significant decrease. Generally, all treatments were caused increase in total free amino acids until the first day of spraying and then occurred decrease even the end of experiment. These results were supported by El-Shehaby and Mohamed (1985) who reported that all fungicides reduced the total free amino acids in onion leaves after 2 and 6 weeks of the last application.

Table 5. Effect of Ridomil plus 50% WP, Vydate 24% SL and their mixture on total free amino acids in tomato leaves at different intervals.

Treatments	Total free amino acids (mg/g dry weight) at different intervals of application			
	1 hour	1 day	7 days	14 days
Ridomil plus 50% WP	$25.13^{b} \pm 0.20$	$23.30^{b} \pm 0.57$	$19.66^{c} \pm 0.61$	$14.33^{ab} \pm 1.20$
Vydate 24% SL	$34.13^{a} \pm 2.60$	$46.86^{a} \pm 4.30$	$23.70^{b} \pm 0.10$	$7.83^{b} \pm 0.23$
Ridomil plus 50% WP + Vydate 24% SL	$24.40^{b} \pm 1.31$	$24.40^{b} \pm 1.31$	$23.70^{b} \pm 0.10$	$15.70^{ab} \pm 0.46$
Control	$24.56^{b} \pm 0.58$	$20.60^{b} \pm 0.87$	$28.53^{a} \pm 1.10$	$24.13^{a} \pm 2.60$
LSD at 0.05	2.54	4.39	1.19	10.30

5. Effect on phenolic compounds:

Analysis of the data showed that the nematicide Vydate had no influence on the phenolic compounds of tomato leaves, as there was no significant difference in the treated and control treatments (Table, 6). Results in the same Table indicated that there were no significant differences between both treatments Ridomil plus and its mixture with Vydate in their effect on phenolic compounds of tomato leaves at 1, 7 and 14 days of application. The Ridomil plus increased phenolic compounds in tomato leaves from 72.84, 75.90, 77.45 and 78.51 in control plants to 78.54, 88.74, 96.08 and 91.11 mg/g dry weight after 1 h. and 1, 7 & 14 days of application, respectively. The corresponding values in the mixture treatment were 81.55, 89.88, 98.68 and 92.89 mg/g dry weight, respectively. These results confirmed that the increase of phenolic compounds in tomato plants due to the fungicide Ridomil plus only.

It has been reported that the treated plants with fungicides suffer from chemical stress (Reid *et al.,* 1992 and Siddiqui 1997). Phenolic compounds, amino acids like proline and flavonoids which might be produced as a result of this stress, may act as protective compounds against pests (Friend, 1977). A correlation has been reported between the phenolic content of healthy tissue and resistance in various host parasite systems like potato/*Verticlium spp.* (McClean *et al.,* 1961) and cotton *Alternaria* spp. (Bashan, 1986 and Prarsad and Lai, 1977).

Table 6. Effect of Ridomil plus 50% WP, Vydate 24% SL and their mixture on phenolic compounds in tomato leaves at different intervals.

Treatments	Total phenolic compounds (mg/g dry weight) at different intervals of application			
	1 hour	1 day	7 days	14 days
Ridomil plus 50% WP	$78.54^{b} \pm 1.12$	$88.74^{a} \pm 0.80$	$96.08^{a} \pm 2.62$	$91.11^{a} \pm 1.99$
Vydate 24% SL	$73.80^{c} \pm 0.93$	$77.56^{b} \pm 0.57$	$79.30^{b} \pm 2.06$	$79.10^{b} \pm 1.01$
Ridomil plus 50% WP + Vydate 24% SL	$81.55^{a} \pm 2.40$	$89.88^{a} \pm 2.03$	$98.68^{a} \pm 0.02$	$92.89^{a} \pm 1.95$
Control	$72.84^{c} \pm 0.95$	$75.90^{b} \pm 1.89$	$77.45^{b} \pm 1.97$	$78.51^{b} \pm 1.70$
LSD at 0.05	2.85	3.06	3.65	3.22

6. Effect on peroxidase enzyme activity

Data in Table (7) indicated that the Ridomil plus, Vydate and their mixture caused significant decreases in peroxidase activity of tomato leaves. There is a gradually relationship between peroxidase activity of tomato leaves and time elapsed in the treatments of Ridomil plus and Vydate. In contrary, the activity of peroxidase enzyme in the mixture treatment was gradually increased with time elapsed. All candidate treatments especially Ridomil plus + Vydate affected the activity of

peroxidase enzyme at the initial time (1h.) and after one day of application as the enzyme activity was sharply inhibited. Peroxidase enzyme can not restored its activity in treated tomato leaves with Ridomil plus, Vydate and its mixture after 14 day of application. Such activity was 71.85, 84.45 and 144.50 with Ridomil plus, Vydate and their mixture treatments, respectively comparing with 241.70 ml OD/Unit/mg protein in control.

These results are agreed with those cited by Ramadan *et al.* (1992) who pointed out that peroxidase activity in profenofos – treated potatoes decreased by 79% comparing with the control. In contrary, Hegazy *et al.* (2006) found that the insecticides, phenthoate, diazinon and chlorpyrifos-methyl increased the activity of peroxidase enzyme in tomato fruits until the fifteenth day of application.

Table 7. Effect of Ridomil plus 50% WP, Vydate 24% SL and their mixture on the specific activity of peroxidase enzyme in tomato leaves at different intervals.

Treatments	Activity of peroxidase enzyme (ml OD/Unit/mg Protein) at different intervals of application			
	1 hour	1 day	7 days	14 days
Ridomil plus 50% WP	$189.03^{b} \pm 5.48$	$124.60^{c} \pm 6.50$	$96.00^{c} \pm 2.82$	$71.85^{d} \pm 1.48$
Vydate 24% SL	$152.73^{c} \pm 4.66$	$131.78^{b} \pm 3.70$	$95.87^{c} \pm 6.833$	$84.45^{c} \pm 0.77$
Ridomil plus 50% WP + Vydate 24% SL	$73.195^{d} \pm 4.122$	$89.7^{d} \pm 5.45$	$136.73^{b} \pm 2.77$	$144.50^{b} \pm 2.85$
Control	$215.53^{a} \pm 0.723$	$205.03^{a} \pm 2.45$	$227.85^{a} \pm 2.47$	$241.70^{a} \pm 10.18$
LSD at 0.05	7.34	6.59	7.38	7.37

From the previous results it is clear that the both pesticides and their mixture affect the biochemical constituents of tomato plants. As the physico-chemical characheristics of the two tested pesticides are different plant reaction to both pesticides could be differed. Consequently, it may be suggested that the synthesis of various metabolic products would also be affected by the application of systemic fungicides and/or nematicides.

Mostly, it could be concluded that spraying tomato plants with the fungicide Ridomil plus, nematicide Vydate and their mixture significantly decreased the tested biochemical constituents of tomato leaves after 14 days of application (end of experiment). Difference cases were observed with the three treatments. Ridomil plus treatment gave significant increase in carotene contents and phenolic compounds. Vydate treatment had no effect on the total soluble sugar and phenolic compounds. The mixture treatment caused significant increase in phenolic compounds and on the

other hand had no effect on carotene contents. Mostly, the effect of the mixture treatment on the tested biochemical constituents of tomato leaves was found in the middle of the side effects of the both tested pesticides. These results led us to recommend the application of the mixture treatment in order to enhance vegetative growth characters and chemical composition of tomato plants via protection of plants against root knot and early and late blight diseases.

Generally, the tested biochemical constituents of tomato leaves can not restored their activity in treated tomato leaves with tested pesticides until the fourteenth day of application. There are low literatures about the side effects of pesticides on the biochemical constituents of tomato. Data presented here can be used as a rough guide to the relative influence of pesticides and their mixtures on the biochemical constituents when each of the tested pesticide is sprayed on vegetable crops at the recommended rate. Further investigations are needed concerning the relation between the changes in biochemical constituents in tomato leaves when pesticides spraying and tomato yield.

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تأثير مبيدي الريدوميل بلاس ٥٠%WP و الفايديت ٢٤%SL " منفردين و مخلوطين على بعض المكونات الكيماوية لأوراق الطماطم تحت الظروف الحقلية

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أجري هذا البحث التطبيقي لدراسة تأثير مبيدي الريدوميل بلاس ٥٠% WP، فايديت ٢٤% SL منفردين أو مخلوطين معاً علي بعض المكونات الكيماوية في أوراق الطماطم خلال فترات زمنية مختلفة بعد المعاملة تحت تأثير ظروف الحقل.

وقد أظهرت النتائج أن المعاملة بمبيد الفايديت أدت إلي حدوث أقصي نقص معنوي في تركيز الكلوروفيل (أ، ب و الكلي) والكاروتين بالأوراق يليها في التأثير المعاملة بمخلوط المبيدين. أدت المعاملة بمبيد الريدوميل بلاس منفرداً إلي نقص معنوي في تركيز الاوراق من كلوروفيل ب والكلوروفيل الكلي فقط. أستخدام التركيز الموصي به من مبيد الريدوميل بلاس أدي إلي زيادة في محتوي الأوراق من كلوروفيل (أ) والكاروتين خلال الفترات المختلفة بعد المعاملة مقارنة بالكنترول. وعلى الجانب الآخر نجد أن مبيد الريدوميل بلاس أدي إلي حدوث نقص معنوي واضح في تركيز السكريات الكلية الذائبة بعد ١، ٧، ١٤ يوم من المعاملة بينما مبيد الفايديت سبب أعلي زيادة معنوية لنفس الصفة بعد ١، ٧ أيام من المعاملة فقط.

هذا وقد أوضحت النتائج أن المعاملة بالريدوميل بلاس والفايديت ومخلوطهما أدي إلي حدوث نقص في تركيز البروتينات الكلية الذائبة بعد ١٤ يوم من المعاملة وعموماً فقد سبب مبيد الفايديت أقل نقص معنوي في هذة الصفة.

أظهرت النتائج إن كل المعاملات أدت إلي زيادة محتوى الأوراق من الأحماض الأمينية الكلية الحرة وذلك في اليوم الأول بعد المعاملة فقط ثم تلي ذلك نقص تدريجي حتى نهاية التجربة. لم يظهر المبيد النيماتودى (فايديت) أي تأثير معنوي علي محتوي أوراق الطماطم من المركبات الفينولية بينما كان تأثير مبيد الريدوميل بلاس معنوياً حيث إنه أدي إلي زياده محتوى الأوراق من المركبات الفينولية من ٧٥,٩٠، ٧٧,٤٥، ٧٨,٥١ ملليجرام / جرام وزن جاف في الكنترول إلي ٨٨,٧٤، ٩٦,٠٨، ٩١,١١ ملليجرام / جرام وزن جاف في الأوراق بعد ١، ٧، ١٤ من المعاملة علي الترتيب.

هذا وقد أحدثت المعاملات نقصا معنوياً علي نشاط أنزيم البيرواكسيديز في أوراق الطماطم ومن الواضح أن معاملة أوراق الطماطم بالمبيدات المختلفة أدت إلى عدم استعادة إنزيم البيرواكسيديز لنشاطه حتي اليوم الرابع عشر بعد المعاملة.