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RESPONSE OF NATURALLY VIRUS INFECTED-TOMATO PLANTS TO YEAST EXTRACT AND PHOSPHORIC ACID APPLICATION.

BY

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ABSTRACT

In field experiments during 1998 and 1999 summer seasons; tomato seedlings c.v. Fakulta were sprayed 5 times in intervals of 10 days with yeast preparation (i.e yeast extract + Vit. E + selenium as antioxidant) in the first experiment meanwhile, other plants were fertilized 5 times at the same intervals of spraying experiment with 5 Kg H_3Po_4 / fed. (P – soil application) in the drip irrigation as a second experiment. Also, a third group of plants in the third experiment were subjected to both foliar spray (as in the first experiment) and P-soil application (as in the second one).

The length of the main stem, number of branches and leaves, leaf area / plant and fresh and dry weights were significantly increased with yeast extract, P-soil application and their combination as well.

Also, the highest percentages of fruit set, fruits number and fruit yield/plant were existed with the combined treatment followed by the P-soil application .

In addition, auxin (IAA) and Gibberellic acid (GA_3) were increased in leaves of treated plants with all applied treatments in contrast with abscisic acid (ABA) that gave the highest amount in the untreated plants (control) compared with different applied treatments.

Moreover, total carbohydrates and phosphorous element reached their maximum concentration in leaves of combined treated plants. In contrast, the crude protein, nitrogen and potassium concentrations which were in their highest levels in leaves of control plants compared with the other applied treatments.

In general, the present study strongly admitted the use of such treatments not only to diminish the deleterious effects of virus infection and increases tomato productivity but also to avoid all cautions about the wide and common insertion of many pesticides in the agricultural system.

INTRODUCTION

In Egypt, tomatoes are the major vegetable crop being grown in several plantings. In recent years, significant losses of tomato fruit yield existed during summer cultivation due to their infection with certain virus diseases. Of these diseases, is the infection of plants with the tomato yellow leaf curl virus (TYLCV). TYLCV is considered one of the limiting factors for both tomato growth and its final fruit production during summer plantings. It was reported as a destructive disease by many workers (e.g Mazyad *et al.*, 1986 and Al-Beshr and Salama, 1989). It causes leaf curling, yellowing and stunting of the plants (Brunt *et al.*, 1990). Its incidence may reach 100 % at the end of the season (Allam *et al.*, 1994).

Because the great current concern about the environment indicates a need to limit application of chemicals for plant disease control. Since, many of pesticides have posed a serious threat to human health as some of them have already been proved to be either mutagenic or teratogenic. Also, a broad and often over use of pesticides is ecologically harmful, toxic to many vertebrates, and may lead to a development of pesticide resistance in the pathogen (Ozeretskovskaya, 1995). Hence, one of the most encouraging and promising techniques for plant protection and increasing productivity is using either natural extracts or a chemical that is not itself a pesticide.

Therefore, the prospect of the present study was to investigate the role of assigned natural yeast extract as spraying material or that of phosphoric acid as soil treatment and their combination on growth, some physiological aspects and productivity of summer tomato plantings grown under field conditions without any pesticides treatment. Thereby, under these conditions , plants, being naturally virus infected.

MATERIAL AND METHODS

Two field experiments were carried out in The Experimental Station at Mansoura during 1998 and 1999 summer seasons. The commercial tomato cultivar, Fakulta (this cultivar is widely cultivated in Egypt during the summer seasons) was the botanical material in this study .

Thirty-five days after sowing seeds; seedlings were transplanted in the field at the first of July in 1998 and 1999 seasons. Tomato seedlings were transplanted, 30 cm. apart on one side of the row of 1 m. wide and 3.5 m. long. The experiment unit area was 16 m^2. Randomized complete block design of 4 replicates was adapted herein. After 10 days of transplanting , plants were treated as follows :

A -Foliar spraying experiment: -

Plants were sprayed 5 times at 10 days intervals with the following mixture: yeast extract of 20 ml/L +1 ml/L. of each of vitamin E and selenium as antioxidant.

Yeast preparation :-

Using a technique allowed pure yeast cells to be grown and multiplied efficiently during favorable aerobic and nutritional conditions. Also, to produce de-novo beneficial bioconstetuents i.e. carbohydrates, sugars, proteins, fatty acids, amino acids, hormones – etc.. Then the growth media was subjected to two cycles of deep freezing and sowing to induce the release of different bioconistituents outside yeast cells .

- Data of yeast extract analysis for different bioconstituents are shown in Table (1).

B -Soil application experiment :-

In this experiment plants were fertilized 5 times with H_3PO_4 in the drip irrigation water with the rate of 5 kg/fedan at the same dates of the spraying experiment .

C-Combined experiment :-

In this experiment tomato plants were subjected to both foliar spraying with the same mixture as in the first experiment and to soil treatment with phosphoric acid as in the second experiment at the same time of application. Twen –20 was added to the foliar spraying mixture at 1 ml/L as spreading agent . All agricultural practices were done according to those recommended for tomato crop .

Experimental parameters :-

(1) **At the middle of harvesting season**: plant height, number of leaves and shoots per plant, fresh and dry weights (gm./plant) as well as leaf area (cm^2) were determined.

(2) **At full blooming stage**: a- in the samples of dry shoots total carbohydrates and NPK concentration were estimated. b- samples of fresh leaves were taken for photosynthetic pigments and endogenous phytohormones determination only in 1999 season.

(3) Number of fruit set/plant, fruits number/plant and fruit yield/plant were recorded in both seasons.

Methods of measuring and estimating of collected data:

a) Plant growth :-

Total leaf area/plant was determined according to the method of Derieux *et al.*, (1973), on dry weight basis .

b) Chemical analysis :

Total carbohydrates, nitrogen, phosphorous and potassium in both tomato leaves and in the yeast extract were determined according to the methods described by Michell *et al.*, (1956) , Horneck and Miller (1998), Sandell (1950), Horneck and Hanson (1998), respectively. Meanwhile, micronutrients and

Table (1): Chemical analysis of yeast extract.

Minerals				Amino acids (mg/100 g dry weight)		Carbohydrates (mg/100 g dry weight)		Enzymes (mg/100 g dry weight)		Vitamins (mg/100 g dry weight)	
Macro (g/100 g dry weight)		Micro (ug/100 g dry weight)									
Total N	7.23	Al	650.2	Arginine	1.99	Carbohydrates	23.2	Cytochrome oxidase	0.35	Vitamin B1	2.23
P_2O_4	51.68	Ba	175.6	Histidine	2.63	Glucose	13.33	Cytochrome peroxidase	0.29	Vitamin B2	1.31
K_2O	34.39	Co	67.8	Isoleucine	2.31			Catalase	0.063	Riboflavin	4.96
		Pb	438.6	Leucine	3.09					Nicotinic acid	39.88
		Mn	81.3	Lysine	2.95					Panthothenic acid	19.56
		Sn	223.9	Methionine	0.72					Biotin	0.09
		Zn	335.6	Phenyalanine	2.01					P-amino benzoic acid	9.23
		NaO	0.35	Thereonine	2.09					Vitamin B6	1.25
		MgO	5.76	Tryptophan	0.45					Folic acid	4.36
		CaO	3.05	Valine	2.19					Thiamine	2.71
		SiO_2	1.55	Glutamic acid	2.00					Pyridoxine	2.90
		SO_2	0.49	Serine	1.59					Vitamin B12	153 ug/100 g)
		Cl	0.06	Aspartic acid	1.33					Inositol	265.1 mg/100 g)
		FeO	0.92	Cystine	0.23						
		NaCl	0.30	Proline	1.53						
				Tyrosine	1.49						

vitamins, amino acids and enzymes in the yeast extract were determined according to the methods described by A.O.A.C.(1990), Rosen (1957) and Maxwell and Bateman (1967), respectively.

c) Photosynthetic pigments and endogenous phytohormones determination: -

At 1999 season during the full blooming stage fresh leaves were taken for photosynthetic pigments and endogenous phytohormones determination. Photosynthetic pigments were determined according to the method described by Nornal (1982). While, the endogenous phytohormones were extracted according to the method described by Sadeghian (1971). Then, the method of Vogel (1975) was used for the methylation of hormones and determined by the Gas Liquid Chromatography (GLC). The GLC analysis was carried out with a dual flame ionization detector.

Statistical analysis:

Data of the present study, were statistically analyzed according to Snedecor and Cochran (1989) and the differences between means were compared using Duncan's multiple rang test (1955) at 5% level of significance.

RESULTS AND DISCUSSION

Growth aspects:

As shown in Table (2) the shortest tomato plants were those untreated–naturally virus infected i.e. the control ones. Meanwhile, plant height was significantly increased with the spraying mixture (i.e. yeast extract, vit. E and selenium) followed by phosphorus –soil application (as H_3PO_4) . Also, the combined treatment (i.e foliar spray + soil application)exhibited the highest values of this parameter. That was true in the two seasons.

Considering plant height as one of the growth parameters, it could be concluded that the infection of tomato plants with the yellow leaf curl virus affected either cell division and/or cell elongation.That was previously indicated as the main symptoms of this virus infection (Brunt *et al.*, 1990 and Allam *et al.*, 1994). Here, it is of interest to note that all applied treatments diminished the inhibitory effect of virus infection upon stem length. In other words, such treatments stimulated cell division and / or cell elongation. So, the results of the present study could attribute this stimulatory effect to the increases in the endogenous levels of growth phytohormones (i.e. auxin and gibberellin) as indicated in Table (5).

As for the number of branches, it could be clearly noticed that foliar spray with the assigned mixture or P- soil treatment or their combination significantly increased this number in naturally virus infected tomato plants when compared with those untreated plants (Table 2). Also, as in case of plant height, the combination treatment was more pronounced in this respect followed by the spraying mixture and the soil application ranked the last in this respect. Such stimulation of branching, as well as other growth characters in tomato plants, in spite of their virus infection, could be strongly correlated with the alteration of hormonal profile.

Table (2): Effect of foliar spraying mixture (yeast extract, vit. E & selenium) and/or P-soil application (H_3PO_4) on some growth characteristics of tomato yellow leaf curl virus (TYLCV) naturally infected plants under field conditions during 1998 and 1999 seasons.

Characters / Treatments	Plant height [main stem] (cm)		Number of branches/plant		Number of leaves/plant		Leaf area (cm^2)/plant		Shoots fresh weight (g/plant)		Shoots dry weight (g/plant)	
	Seasons											
	1998	1999	1998	1999	1998	1999	1998	1999	1998	1999	1998	1999
Spraying Mix.	76.00 ^{C}	78.33 ^{C}	10.00 ^{B}	11.33 ^{B}	109.67 ^{B}	128.67 ^{C}	20395 ^{B}	18839 ^{BC}	930.00 ^{B}	823.33 ^{C}	99.00 ^{BC}	110.93 ^{B}
P- Soil appli.	84.67 ^{B}	87.67 ^{B}	8.67 ^{C}	9.33 ^{C}	114.67 ^{B}	153.00 ^{B}	20817 ^{B}	21803 ^{B}	963.33 ^{B}	946.67 ^{B}	122.42 ^{B}	125.25 ^{B}
Spraying + soil appli.	91.67 ^{A}	96.33 ^{A}	12.67 ^{A}	14.33 ^{A}	150.66 ^{A}	195.00 ^{A}	31283 ^{A}	31366 ^{A}	1033.33 ^{A}	1200.00 ^{A}	170.47 ^{A}	161.49 ^{A}
Control	70.67 ^{D}	74.00 ^{D}	8.33 ^{C}	8.33 ^{C}	84.00 C	94.67 ^{D}	16938 ^{C}	15867 ^{C}	523.33 ^{C}	556.67 ^{D}	67.31 ^{C}	88.50 ^{C}

With regard to the number of leaves and the leaf area / plant, it could be noticed that during 1998 and 1999 seasons, these two-parameters were significantly increased with foliar spray of spraying mixture, P- soil application and their combination in ascending order .

The increasing in leaves number as well as their area could be reflected into vigorous growth of tomato plants despite of their virus infection. Hence, the photosynthetic area was increased and that could be reflected upon the efficiency of photosynthesis process itself. Supporting this opinion are the works of Hopkins (1995) and Hendrix (1995).

Furthermore, the previous mentioned enhancement of plant growth was extended to both fresh and dry weights of treated plants. That means that the treated tomato plants are not only being more able to prevail or to compete the virus infection but also accumulated more dry matter compared with untreated – naturally virus infected plants.

Effects on some bioconstituents and NPK contents :-

Data in Table (3) clearly indicate that the virus infection of tomato plants severely decreased the carbohydrate concentration in the infected leaves . Meanwhile, all applied treatments enabled the plants to accumulate more carbohydrates in leaves, especially in the combined treatment. This enhancement of carbohydrates accumulation reached 338.5 mg/g d.w. , meanwhile it was 87.3 mg/g d.w. in case of the untreated –virus infected treatment.

Nitrogen and the calculated crude protein concentration were higher in the untreated – virus infected plants than in leaves of all other applied treatments. That may be attributed to the protein of virus molecules itself. With regard to the phosphorus concentration, it was only increased in case of combination treatment but was decreased in both spraying mixture and P-soil application treatments as compared with the untreated treatment. Meanwhile, potassium concentration was nearly decreased with different applied treatments and its maximum reduction existed in P-soil application treatment.

Table (3): Effect of foliar spraying mixture (yeast extract, vit. E & selenium) and/or P-soil application (H_3PO_4) on total carbohydrates (mg/g dry weight), crude protein and NPK contents in leaves of tomato yellow leaf curl virus (TYLCV) naturally infected plants during 1999 season.

Characters / Treatments	Total Carbo-hydrates	%			
		Crude protein	N	P	K
Spraying Mix.	275.8 ^{B}	28.19	4.51 ^{C}	0.25 ^{C}	1.89 ^{A}
P- Soil appli.	172.7 ^{C}	26.43	4.23 ^{D}	0.22 ^{D}	1.51 ^{C}
Spraying + soil appli.	338.5 ^{A}	34.80	5.56 ^{B}	0.31 ^{A}	1.73 ^{B}
Control	087.3 ^{D}	39.56	6.33 ^{A}	0.27 ^{B}	1.91 ^{A}

In addition the application of phosphoric acid as soil treatment, enables tomato plants to attain their vigorous growth; since, phosphorous (as inorganic components) is involved in synthesis of ATP (that required for both virus and the host plant as well) the main and unique energy constituent in plant tissues as well as in formation of RNA (that is necessary also for virus replication) and phospholipids. Thereby, phosphorous directly enhance and controls many biosynthesis processes, e.g. carbohydrate and sugar formation , nucleic acids, enzymes and phytohormones (Li, 1985 and Yelenosky, 1985). These bioconstituents and metabolic changes were suggested to be tightly associated with the vigorous growth of the host plants as well as with the virus multiplication (Devlin and Witham , 1983) .

Effect on the photosynthetic pigments :

Data in Table (4) clearly indicate that all the applied treatments increased chlorophyll's (a & b) in leaves of the treated plants compared with that of control leaves during 1999 season. Also, the combined treatment (i.e. spraying Mix + P-soil application) was more pronounced in this respect. Since , chlorophyll's concentration in its leaves was more than three times (1.378 mg/g f.w) of the control leaves (0.417 mg/g f.w.), that could be considered not only the main reason for the obtained growth enhancement and dry matter accumulation (Table. 2) but also extended to attain the highest fruit yield (Table. 6).

Table (4): Effect of foliar spraying mixture (yeast extract, vit. E & selenium) and/ or P-soil application (H_3PO_4) on chlorophyll a & b and carotenoids (mg/g fresh weight) in leaves of tomato yellow leaf curl virus (TYLCV) naturally infected plants under field conditions during 1999 season.

Ch aracters / Treatments	Chl. a	Chl. B	Ch a + b	Carotenids
Spraying Mix.	0.263	0.420	0.683	0.891
P- Soil appli.	0.296	0.310	0.606	0.762
Spraying + soil appli.	0.485	0.893	1.378	0.611
Control	0.211	0.206	0.417	1.195

On the other hand, carotenoids concentration was decreased with different applied treatments comparing with the untreated plants. Also, it could be noticed that the combination treatment was more effective regarding the reduction of carotenoids concentration. Meanwhile, the lowest reduction was existed in case of spraying mixture treatment. That also could considered as a reflection of appeared symptoms of virus infection. Since, virus infected tomato plants without any of the applied treatment were less in their green colour .

Effect on endogenous phytohormones :-

As shown in Table (5) both endogenous auxin (IAA) and Gibberellic acid (GA_3) were clearly increased with the different applied treatments. The greatest concentration of IAA (340ng/g f.w) was existed in the case of foliar

spray with the spraying mixture followed by the combination treatment while the soil application ranged the last in this respect.

Table (5): **Effect of foliar spraying mixture (yeast extract, vit. E & selenium) and/or P-soil application (H_3PO_4) on endogenous auxins (IAA), gibberellin (GA_3) and abscisic acid (ABA) (ng/g fresh weight) in leaves of tomato yellow leaf curl virus (TYLCV) naturally infected plants under field conditions during 1999 season.**

Characters / Treatments	Auxin (IAA)	% as related to the control	Gibberellic acid (GA_3)	% as related to the control	Abscisic acid (ABA)	% as related to the control
Spraying Mix.	340	288	110	138	37	71
P- Soil appli.	124	105	120	150	42	81
Spraying + soil appli.	220	186	140	175	30	58
Control	118	100	80	100	52	100

As for gibberellic acid (GA_3) its greatest concentration was obtained in case of the combination treatment (140 ng/g f.w.) followed by soil application treatment, while spraying mixture ranked the last in this respect .

With regard to the endogenous abscisic acid (ABA) , it was completely irreversibly behaved when compared with the auxin and gibberellin since its highest value (52 ng/g f.w.) was existed in case of untreated –naturally virus infected tomato plants . Meanwhile, the combination treatment exhibited its lowest value .

The previous mentioned results are of great importance. Since, these phytohormones represent the main factors are determining the growth behavior (Devlin and Witham ,1983 and Noggle and Fritz, 1992).

Also, it could be noticed that the plenty of IAA and GA_3 in the treated plants significantly enhanced their vegetative and reproductive growths Tables (2, 4 & 6). In this respect -for example- plant height, number of branches , number and area of leaves and shoots fresh and dry weights Table (2) as well as the endogenous gibberellic acid level Table (5); all of these parameters and their maximum values were existed in case of the combination treatment. The same treatment exhibited the lowest abscisic acid cocentration as compared with control or even other applied treatments. The role of exogenous plant growth regulators especially auxin upon the stimulation of tomato growth and productivity has been previously reported by Hathout *et al.*, (1993). They reported that auxin (IAA) increased the length of the main stem, activated the uptake of some elements and induced flower and fruit giving in number and weight. Also, of interest to note is that, abscisic acid Table (5) and carotenoids Table (4) both are in intimate correlation. Since, their highest values were existed in untreated – naturally virus infected - tomato plants. That is true when compared with the all applied treatments. Moreover, increment of these two

bioconstituents could be considered the main factor for the yellowish colour that charactarizes the untreated – naturaly virus infected plants .

Fruiting and fruit yield :

As shown in Table (6) spraying mixture, P- soil application and their combination significantly increased the percentages of fruit set, fruit number and fruit yield per plant comparing with the untreated plants during the two seasons. Also, of interest is that the combination of the two applied methods was more effective regarding the all estimated producing parameters . Since, each of these parameters in the combination treatment was increased more than two times of the untreated treatment (control).

Table (6): Effect of foliar spraying mixture (yeast extract, vit. E & selenium) and/or P-soil application (H_3PO_4) on fruit setting, number and fruits yield of tomato yellow leaf curl virus (TYLCV) naturally infected plants under field conditions during 1998 and 1999 seasons.

Characters / Treatments	% of fruit set/plant		Fruits number/plant		Fruit yield g/plant	
	Seasons					
	1998	1999	1998	1999	1998	1999
Spraying Mix.	28.00 ^{C}	30.67 ^{C}	27.00 ^{C}	31.33 ^{B}	3785.80 ^{B}	3466.67
P- Soil appli.	31.67 ^{B}	33.67 ^{B}	32.33 ^{B}	34.33 ^{B}	3583.33 ^{B}	3800.00
Spraying + soil appli.	40.33 ^{A}	43.33 ^{A}	39.00 ^{A}	43.33 ^{A}	4400.00 ^{A}	4766.67
Control	17.00 ^{D}	19.00 ^{D}	16.67 ^{D}	18.00 ^{C}	1850.00 ^{D}	1866.67 ^{DC}

Here, of interest is that the enhancement of vegetative growth (Table, 2) prolonged to the reproductive one (Table, 6) that led to attain the highest fruit yield of treated tomato plants comparing with untreated – naturally virus infected - tomato plants .

So, plenty of these bioconstituents could minimize or diminish the deleterious effects of virus infection . Because under such treatments virus requirements could be provided by plants with less injury effects upon growth and yield of host plant.

In general, it could be concluded that the spraying mixture as including yeast extract which represent a rich source of phytohormones [Barnett *et al.*, (1990), Fathy and Farid, (1996), Atawia and El-Desouky, (1997) and Fathy *et al.*, (2000), [see also Table (2),] increased the ability of tomato plants to grow well in spite of the virus infection conditions, Tables (4, 5 & 6) . These effects mainly minimizing all the injury aspects of virus infection in diseased plants . Also , it could be enabled the plants to synthesize more photosynthates (Table, 3) and probably roots to uptake more nutrients, especially when the increases of endogenous phytohormones are considered (Table, 5) .

Finally, it could be concluded that yeast extract and phosphorous treatments upon naturally – virus infected tomato plants not only enables plants to attain their vigorous growth and minimizing deleterious effects of virus infection but also extend to improve the final tomato fruit yield. Therefor, the present study strongly admit the use of such treatments not only to diminish virus injuries and increase fruit yield but also to avoid all cautions about the use of pesticides those are widely agriculturally applied for control of virus transmitants such as the white fly in case of tomato plants.

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استجابة نباتات الطماطم والمصابة طبيعيا بالفيروس للمعاملة بمستخلص الخميرة وحمض الفوسفوريك

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في تجارب حقلية خلال الموسمين الصيفيين ١٩٩٨ ، ١٩٩٩ عوملت نباتات الطماطم صنف فاكولتا على النحو الأتي بعد شتلها بعشرة أيام

١- نباتات تم رشها بمستخلص الخميرة مضافا إليه فيتامين E والسيلينيوم كمادة مضادة للأكسدة وذلك ٥ مرات بفاصل عشرة أيام بين كل رشة والتالية .

٢- نباتات أخرى تم إمدادها في ماء الري (بالتنقيط) بحمض الفوسفوريك بمعدل ٥ كجم/للفدان وذلك خمس مرات فى نفس مواعيد الرش فى المجموعة الأولى.

٣- المجموعة الثالثة من النباتات تم معاملتها بالرش كما فى المجموعة الأولى بالإضافة إلى المعاملة الأرضية كما فى المجموعة الثانية بالإضافة إلى نباتات المقارنة والغير معاملة بأى من المعاملات السابقة .

وكانت أهم النتائج المتحصل عليها كالأتي :

- زاد معنويا كل من : ارتفاع النبات ، عدد الأفرع الجانبية ، عدد الأوراق ومساحتها والوزن الرطب والجاف للمجموع الخضري وذل بمختلف المعاملات.
- وأيضا كانت أعلى زيادة معنوية فى كل من عقد الثمار ، عدد الثمار وكذلك محصول الثمار للنبات فى المعاملة المشتركة (الرش + معاملة التربة) يليها معاملة التربة فقط .
- وكذلك زادت كمية الأكسين والجيبريلين الداخلية فى أوراق جميع المعاملات على العكس تماما كانت كمية حمض الأبسيسيك الداخليةحيث كانت أعلى قيمة لها فى أوراق النباتات الغير معاملة (المقارنة) عن جميع المعاملات المتبعة.
- وكان أعلى مستوى للكربوهيدرات الكلية وكذلك عنصر الفوسفور فى أوراق نباتات المعاملة المشتركة – وعلى العكس من ذلك كان محتوى الأوراق من البروتين الخام ، والنيتروجين والبوتاسيوم أعلى فى أوراق النباتات الغير معاملة عنه فى أوراق نباتات المعاملات المختلفة
- وقد أستخلص من هذه الدراسة إمكانية استخدام مثل هذه المعاملات ليس فقط لتقليل مضار الإصابة الفيروسية وزيادة محصول الثمار بل أيضا لتجنب كل المحاذير حول الاستخدام الشائع لكثير من المبيدات الحشرية على مثل هذه المحاصيل والتي تستهلك ثمارها طازجة.