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IMPACT OF BIOAGENTS AND ORGANIC ACIDS ON ALTERNARIA SOLANI, THE CAUSAL ORGANISM OF TOMATO EARLY BLIGHT DISEASE AND THEIR SIDE EFFECT ASSESSMENT.

Moustafa M. S. H., Asmaa M. A. ALkolaly and Hala A. M. El-Dakar
Plant Pathol. Res. Inst., A.R.C., Giza,
Egypt.

ABSTRACT

Pesticides have a specific purpose in society; they are intended to control insects, fungi, bacteria and weeds that damage crops; however, pesticides can adversely affect some nontarget living organism. Even the least-toxic products, and those that are natural or organic products, can cause human health problems. In this work, we studied the effect of Bio Arc (*Bacillus megaterium*) a biocide, the resistance inducer "salicylic acid" and the growth promoter "humic acid" were studied on *Alternaria solani* the causal of tomato early blight disease, compared with the recommended biocide, Bio Zeid (*Trichoderma album*). Since the most of tomato fruits are permanently consumed fresh, therefore it was very important to assess the possible toxicological risk of long-term consumption of tomato fruits harvested from plants treated with any of the aforementioned biocides. In *vivo* study, Bio Arc was the most effective one (67.8%) followed by Bio Zeid (43%) then salicylic acid (40%), in contrast to humic acid, which resulted in very low efficacy (35.4%). However, while disease severity on plants treated with Bio Zeid increased gradually tell after the third spray, on plants treated with Bio Arc and salicylic acid increased gradually tell the second spray. feeding rats for 4 weeks on fodder, mixed with juice of tomato fruits harvested from plants treated with salicylic acid,Bio Zeid or Bio Arc compared with two control treatment (control+) and (control-) revealed that: in case of salicylic acid, no significant adverse effect was noticed on Alanineaminotransferase (ALT), High density lipoprotein (HDL) and albumin; in contrast to Aspertateaminotranferase (AST), cholesterol, triglyceride, Low density lipoprotein (LDL), creatinin and urea which were significantly increased .BioZeid showed no significant adverse effect on AST, cholesterol, HDL or albumin; in contrast to ALT, triglyceride, LDL, creatinin and urea .BioArc showed no significant adverse effect on ALT and AST, on the other hand, the levels of cholesterol, triglyceride, LDL, creatinin and urea significantly elevated. In contrast, HDL and albumin which significantly decreased. Elevation of AST and ALT in case of salicylic acid,Bio Zeid respectively indicated that, the rats liver may be hurt, especially liver weight of rats fed on fodder mixed with tomato treated with Bio Zied significantly decreased. Raised of creatinin and urea level in blood of rats fed on fodder mixed with tomato treated with salicylic acid ,BioZied or Bio Arc indicating that the rats kidneys may be adversely affected, especially kidney weight of rats fed on fodder mixed with tomato treated with Bio Zied significantly decreased.

Keywords: Biocides, BioZied, BioArc, Salycalicacid, Humic acid, toxicological studies.

1. INTRODUCTION

Tomato (*Lycopersicom esculentum*) is the second most important vegetable crop next to potato. Global tomato production is currently around 130 million tons, of which 88 million are destined for the fresh market and 42 million are processed (Anonymous, 2016).In 2014 Egypt ranks amongst world tomato-producing countries, with estimated production of 8,288,043 tons (anonymous2014). Tomato plants are subject to many destructive diseases, i.e. Early blight and Collar rot, caused by the fungus *Alternaria solani,* Late blight, caused by the fungus *Phytophthora infestans,*Septoria leaf spot, caused by the fungus *Septoria lycopersici*, Anthracnose, caused by the fungus *Colletotrichum coccodes*, and Powdery mildew, caused by the fungus *Oidium neolycopersici* (Gleason and Edmunds,2006).

These diseases can cause economically important symptoms on seedling, foliage and fruit. Yield losses up to 79% from Early blight damage have been reported from Canada, India, the United States, and Nigeria, while Collar rot can cause seedling losses of 20% to 40% in the field (Rein and Vpprrips, 2006). Fungicide treatments were generally the most effective control measures, but intensive use of toxic pesticides in agriculture worldwide has raised serious concerns about health issues, and environmental pollution specially, after (Carson 1962) published her book "Silent Spring". In addition, many fungi could develop resistance races to most of the used fungicides. Under the above circumstances, it becomes inevitable to develop bio-based, eco-friendly, biodegradable plant derived pesticides, or microbial pesticides, in order to control plant pathogens. Biological control or bio-control using antagonistic microorganisms, resistance inducers and growth promoters offer a practical and economical alternative for the management of plant pathogens (Chandrashekara, *et. al*., 2012).Soil drench with furfural, humic, folic acid and the bioagents *T. harzianum*, *T. viride*, *Bacillus subtilis* and *Pseudomonas fluorescens* resulted in significant control the pathogenic fungi, *Fusarium solani*, *F. oxysporum*, *Rhizoctonia solani*, *Sclerotium rolfsii*, *Sclerotinia sclerotiorum*, *S.minor*, *Macrophomina phaseolina*, *Alternaria solani and Pythium* sp. on cucumber, cantaloupe, pepper and tomato in pot experiments (Abdel-Kader, *et al*., 2012). On the other hand, salicylic acid significantly reduced severity of root rots of tomato plants caused by *Rhizoctonia solani*, *Fusarium solani* and *Sclerotium rolfsii* under greenhouse and field conditions, at the same time, it resulted in significantly increase of all growth parameter such as number of branches, leaves per plant, leaf area and leaves dry weight (El-Mohamedy, *et al*., 2014).Bio Zeid 2.5% is recommended to control the early blight disease on tomato in Egypt (Anonymous, 2015). On the other hand, the commercial biological control agents BioZeid and BioArc, and salicylic acid (chemical resistance inducers) significantly reduced the disease severity of cucumber powdery mildew, caused by *Sphaerotheca fuliginea* (Awad, *et. al*, 2012).

This work is aiming to study the effect of the biocide BioArc, the resistance inducer salicylic acid and the growth promoter humic acid on *Alternaria solani* the causal of tomato early blight disease, compared with the recommended biocide "BioZeid". In addition, since the most of tomato fruits are permanently consumed fresh, therefore it was very important to assess the possible toxicological risk of long-term consumption of tomato fruits harvested from plants treated with any of the aforementioned biocides.

2. MATERIALS AND METHODS

In *vivo* studies:

Under greenhouse, five weeks old tomato transplants (Vr. Castel rock) were planted in pots (50Ǿ cm) containing loam sandy soil; four transplants in each. The pots were divided into five groups, each group 15 pots (3replicates, each replicate 5 pots). The pots were arranged in complete randomized block design. All the plants received all the recommended practices, then the plants groups were sprayed with one of, Bio Zeid (*Trichoderma album* 2.5% w/w 10 millions spores/g), Bio Arc (*Bacillus megaterium*, 6% w/w contains 25 millions cells /g), salicylic acid or humic acid, separately at the rate of 2.5 g/l, 2.5 g/l, 4 g/l and 4 g/l water, respectively, the second spray was two days before infection. When the plants good established, they were artificially infected, since they were sprayed with spore suspension (1 x 10^5 spore/ml) from *Alternaria solani* (*Alternaria solani* culture was kindly submitted from department of Plant Diseases and Fungi Survey and Identification Research;Plant Pathology Research Ins, ARC.)that was grown on V8 juice agar-CaCO3media 6.5 pH, at 25±1 C^o, with alternatively 12 hours periods of light and darkness, (Tatiana *et.al*, 2010). In order to provide adequate environment for the disease establishment, the floor of the greenhouse was regularly sprayed with water. The fifth group was sprayed with water only to serve as control. Each group was sprayed three times in 2 weeks intervals after the infection (the plant foliage was spayed tell the beginning of runoff point. Disease incidence and disease severity were recorded 2 weeks after each application. Disease incidence was assessed using a scale consisted of six categories, ranged from 0 to five (0 = no infection, 1 = scattered spots of infection less than 10% of the leaf area, 2 = 10% and >20%, 3 = 20% and >30%, 4 = 30%, and >40% and 5 = < 40% of the leaf area; then disease severity was calculated using the equation developed by (Towsend and Heuberger 1943), in which:

$$DS\ (\%) = \Sigma(nv) / NV \times 100$$

Where,

n = degree of infection according to the scale.

v = number of sample per category.

V = total number of sample screened.

N = highest degree of category.

Then efficacy of fungicides was calculated using Abbott's formula (Abbott, 1925):

$$Efficacy\ (\%) = (X - Y) / X * 100$$

Where,

X = disease severity in the control.

Y = disease severity in the treatment

Two weeks after the third application, area under disease progress curve, during the application period, was calculated using the following equation, according to (Campbell and Madden1990):

$$AUDPC = \sum_{i=1}^{n-1} \frac{Yi+Y(i+1)}{2} \mathrm{x}\ (t_{i+1} - t_i)$$

Since:

AUDPC is the area under disease progress curve

Yi is the disease severity assessed at the beginning of treatment and after two weeks from each treatment

Ti is time (two week for each disease severity assessment)

n is the total number of observations (4).

Disease severity data were statistically analyzed as complete randomized block design and least significant differences (L.S.D 0.5) was calculated according to (Fisher, 1948) and (Snedecor and Cochran 1967) and Multiple range and multiple F test (Duncan, 1955), using Web Agri Stat Package computer program (WASP).

Toxicological studies:

Tomato fruits from each treatment were separately collected in clean polyethylene bags; the bags were closed, marked up and kept in deep freeze -2^{o}C to be used in the toxicological studies. The tested animals involved in this study were obtained from Experimental Animals House of Food Technology Research Institute, Agricultural Research Center, Giza, Egypt (ARC). Twenty male Sprague-Dawley albino rats four weeks old, weighting (100 to 120 g), were used in this toxicological study. The rats were kept for adaptation period of two weeks long; feeding on basal diet consisted of corn starch 70%, casein 10%, corn oil 10% ,cellulose 5%, salt mixture 4% and vitamin mixture 1% as described by (Peter and Person 1971). After the adaptation period, the rats were divided into 5 groups (4 rats each). The first group fed for 28 days on basal fodder mixed with 10% juice of tomato fruits, collected from the plants which previously treated with BioZeid. The second group fed for the same period on basal fodder mixed with 10% juice of the tomato fruits, collected from the plants which previously treated with BioArc. The third group fed for the same period on basal fodder mixed with 10% juice of tomato fruits, collected from the plants which previously treated with Salicylic acid. The fourth group fed for the same period on basal fodder mixed with 10% juice of tomato fruits collected from the plants which were not treated with any biocides or salicylic acid (positive Control).The last group of rats fed for the same period on basal fodder only (negative control).At the end of the feeding period, blood samples were collected from each rat separately from the *Retro-Orbital Venous Plexus* in centrifuge tubes by carefully inserting capillary tube 15 – 30 angel near the noise of the rat down to up. The serum was separated and kept at-2^{o}Cfor analysis; then the weight rat's body, brain, liver and kidney were registered for each rate separately. All the biochemical analysis in this work were carried out using spectrum diagnostic kits produced by MDSS GmbH (Hannover, Germany) which were supplied by the Egyptian Company for Biotechnology and according to the methods described in user manual provided with each kit using JENWAY 6400 Spectrophotometer as follow: Aspartateaminotransferas (AST) and Alnineaminotransferase (ALT) activities were colorimetrically determined according to the method of (Bergmeyer and Harder 1986) which depending on the reaction with α-oxoglutrate to produce oxaloacetate and pyruvate, which can be determined spectrophotometrically in the form of hydrazone, which is produced by reaction with 2,4-dinitrophenylhydrazine in an alkaline medium.

Total serum cholesterol (high density lipoprotein cholesterol HDL, low density lipoprotein cholesterol LDL and triglycerides) were determined as follow: Cholesterol was determined using CHOD-PAP–enzymatic colorimetric method which depending upon the reaction of cholesterol esterase with the reagent (IFUFCC08) to produce cholesterol + fatty acids that in turn transformed to Quinoneimine dye. High-density lipoprotein (HDL) was determined using precipitation method in which the LDL and VLDL in sample precipitate with phosphortungstate and magnesium ions; after precipitation the HDL fraction, which remain in the supernatant is determined using the reagent (IFUFCC26). Triglycerides were determined using GPO-PAP-enzymatic method, which depend upon the series reactions with the reagent (IFUFCC39) to form at the last step Quinoneimine dye.

LDL was calculated according the equation

$$\text{LDL} = \text{cholesterol} - \{\frac{\text{triglycerides}}{5} + \text{HDL}\}$$

Urea was assessed using urease-colorimetric method by reaction of the sample with the reagent (IFUFCC40) to hydrolyze the urea in the presence of water and urease to produce ammonia and carbon dioxide.

Creatinine was determined using the Puffered Kinetic jaffe reaction without deproteinization method, using the reagent (IFUFCC09) in which creatinine react with picric acid under alkaline condition to form a yellow-red complex. Albumin was determined using modified bromocresol green methods which based on its binding with the reagent bromocresol green (IFUFCC52) in 4.1 pH

Statistical analysis

The analysis of variance (ANOVA) was performed then critical values of Duncan's new multiple range test (Duncan, 1955) was calculated and date were represented as mean ± SEM (standard error of the mean) and category letter

3. RESULTS

In *vivo* studies:

Treating tomato plants with two biocides Bio Zeid and Bio Arc, and two organic acids, salicylic and humic acids, under greenhouse conditions three times after the infection and assess the disease severity and disease control efficiency, after one week from infection (Table 1) indicated that, after the first week plants treated with BioArc showed the least disease severity (10.0%) with

52.4% disease control efficacy, followed by BioZeid with 13.0% disease severity and 38%disease control efficacy. No significant difference was recorded between them. Salicylic acid recorded 16.0% disease severity and 23% disease control efficacy with significant difference between it and the two biocides. No significant difference between Humic acid and the control plants was found. After the second week, there were significant differences among all treatment. Bio Arc still the most effective on recording 18% disease severity and 55% disease control efficacy, followed by BioZeid with 23 % disease severity and 42.5% disease control efficacy; then Salicylic acid with 27% disease severity and 32.5% disease control efficacy. Humic acid was the least effective one recording 33% disease severity and 17.5% disease control efficacy. After the fourth week, all the treatment were in the same order , since BioArc recorded 67.8% disease control efficacy followed by Bio Zeid, salicylic acid then humic acid with only 35.4% disease control efficacy. Worthwhile, however disease severity in case of Bio Zeid increased gradually, disease severity in case of Bio Arc, salicylic acid and humic acid increased after the second spray then decreased after the third spray (fig.1). Calculation of area under disease curve (Table 1) indicated that, there were significant differences among all the tested material. Disease severity ranged from 26.04 to 31.3% compared with 37% in control treatment. Bio Arc was the superior with 26.04% disease severity and 28.5% disease control efficacy, followed by Bio Zied with 27%disease severity and 27% disease control efficacy, then salicylic acid with 30.68%disease severity and 17.3% disease control efficacy. Humic acid was the least effective one resulting in 31.3% disease severity and only 15.4% disease control efficacy, compared with 37% disease severity in control treatment.

Table 1: Early blight disease severity, disease control efficacy and area under disease progress curve on tomato plants sprayed three times with BioZeid, BioArc, Salicylic acid and Humic acid.

Treatments	After 1st week from infection		After 2nd week from infection		After 3rd week from infection		After 4th week from infection		AUDPC	
	DS	Eff	DS	Eff	DS	Eff	DS	Eff	DS	Eff
Bio Zeid	13b	38	23c	42.5	28c	51.7	37c	43	27	27
Bio Arc	10b	52.4	18c	55	24d	58.6	21d	67.8	26.04	28.5
Salicylic acid	16b	23	27b	32.5	40c	31	39bc	40	30.68	17.3
Humic acid	21a	0.0	33b	17.5	45b	22.4	42b	35.4	31.3	15.4
Control	21a		40a		58a		65a		37	
LSD 0.05	1.89		4.05		2.83		1.84			

DS =disease severity
Eff = disease control efficacy
AUDPC = Area under disease progress curve

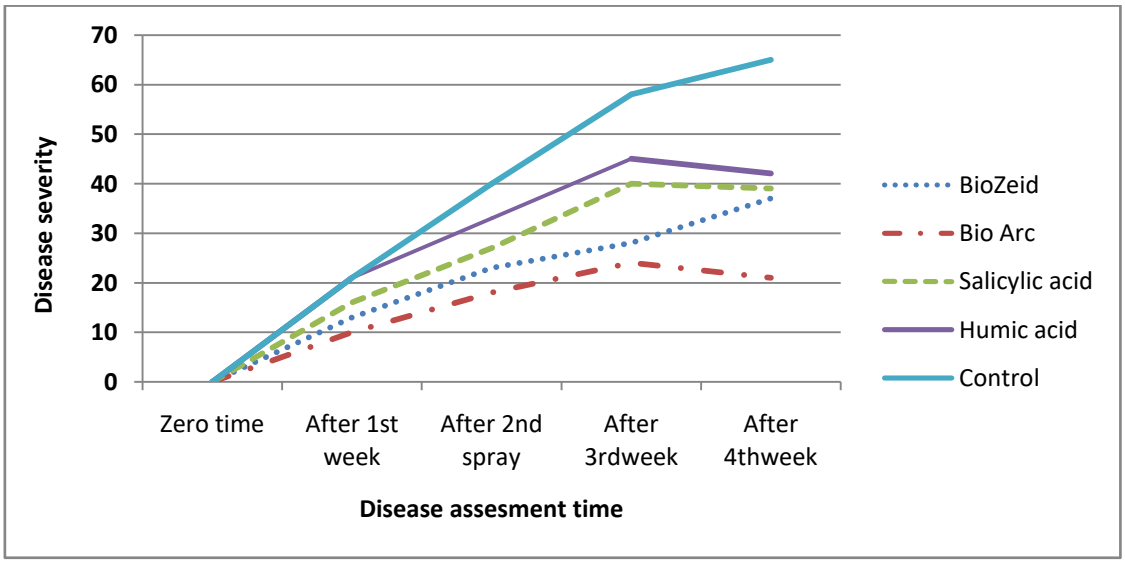

Figure 1: Early blight disease severity development after three application of Bio Zeid, Bio Arc, Salicylic acid and humic acid.

Toxicological study:

Data in Table (2) indicating that Alanineaminotransferase (ALT) level, blood of rats fed on fodder contained 10% juice of tomato fruits harvested from plants treated with BioZeid, since ALT was significantly increased. Level of ALT in blood of rats fed on fodder contained 10% juice of tomato fruits harvested from plants treated to salicylic acid or BioArc did not show any significant difference compared with tomato fruits harvested from the plant of the two control treatments (control+ and control-). According to Aspartateaminotransferase (AST), feeding rates on fodder contained 10% juice of tomato fruits harvested from plants treated with salicylic acid resulted in significant higher AST. No significant different was found between the blood serum of rats fed on fodder contained 10% juice of tomato fruits harvested from plants treated with BioArc or BioZeid and the two control treatments (control+ and control-). Concerning total cholesterol, salicylic acid or BioArc in rats fodder resulted in significant increase of total cholesterol with 120.25 and 114.25 mg/dl respectively compared with BioZeid, control+ and control- with 108.5, 99.25 and 103.75 mg/dl respectively. On the other hand, tomato juice contaminated with Salicylic acid resulted in the highest significant increased triglyceride with 147.25 mg/ld, followed by BioArc, and BioZeid with 113.5 and 122.25 mg/ld respectively; compared with the two control treatments (control+ and control-) which recorded 78.75 and 86.25 mg/ld respectively. Regarding HDL, feeding rates on fodder mixed with juice of tomato fruits harvested from plants treated with Bio Arc showed significant decrease of HDL content in blood serum (51.5 mg/ld) compared with Salicylic acid and Bio Zeid, that did not affect the HDL content in blood serum (56.0 and 56.6mg/dl respectively) on one side, and the two control treatments (55.56 and 58.25 mg/dl respectively) on the other hand. Relating to LDL the three treatments Salicylic acid, Bio Arc and Bio Zeid resulted in significant increased LDL content (39.75, 38.0 and 40.75 mg/dl respectively) compared with the two treatments control+ and control- they recorded 24.0 and 25.75 mg/ dl respectively. Regarding to Albumin, feeding rates on fodder mixed with juice of tomato fruits harvested from plants treated with Bio Arc showed significant decrease of Albumin content in blood serum (2.35 mg/dl) compared with the all other treatments. Salicylic acid or BioZeid recorded 4.4 and 3.325 md/dl respectively and the two control treatments, control+ and control-, they recorded 4.5 and 3.725 mg/ld respectively.Respecting creatinin, all the treatments, salicylic acid BioArc and BioZeid resulted in significant increase of creatinin in blood serum of the experimental rats (0.293, 0.298 and 0.313 mg/ld respectively), compared with control treatments, control+ and control-, those recorded 0.285 and 0.220 mg/dl respectively. All the three treatments, Salicylic acid, Bio Arc and Bio Zeid, recorded significant increased level of urea in blood serum, with 14.5, 13.75 and 14.5 mg/dl respectively; compared with the control treatments, those recorded 12.0 and 11.5 mg/dl respectively.

Table 2: Blood chemoanalysis and body-, brain-, liver- and kidney- weight of rats fed on fodder mixed with tomato fruits juice harvested from plants treated with salicylic acid, Bio Zied , Bio Arc and the two control treatments (control+ and control-) respectively.

Analysis test	Salicylic acid	BioZied	Bio Arc	Control +	Control -
ALT U/l	17.5±0.75^{b}	36.0±1.06^{a}	22.25±5.80^{b}	21.0±0.89^{b}	21.0±0.0^{b}
AST U/l	82.4±3.07^{a}	57.55±5.91^{b}	44.8±1.60^{b}	56.03±2.15^{b}	56.03±1.50^{b}
Cholesterol mg/Dl	120.25±10.21^{a}	108.5±6.03^{b}	114.25±0.24^{a}	99.25±8.10^{b}	103.75±3.3^{b}
Triglyceride mg/Dl	147.25±5.92^{a}	113.5±1.35^{b}	122.25±7.04^{b}	78.75±3.08^{c}	86.25±3.54^{c}
HDL mg/Dl	56±3.08^{a}	56.6±1.30^{a}	51.5±1.29^{b}	55.56±0.56^{a}	58.25±0.74^{a}
LDL mg/Dl	39.75±2.30^{a}	38±1.54^{a}	40.75±5.34^{a}	24±0.50^{b}	25.75±1.08^{b}
Albumin mg/Dl	4.4±0.37^{a}	3.325±0.09^{a}	2.35±0.26^{b}	4.05±0.39^{a}	3.725±0.24^{a}
Creatinin mg/Dl	0.293±0.013^{b}	0.298±0.008^{b}	0.313±0.008^{a}	0.235±0.005^{c}	0.220±0.006^{c}
Urea mg/Dl	14.5±0.25^{b}	13.75±0.25^{b}	14.5±0.22^{b}	12.0±0.35^{a}	11.5±0.25^{a}
Body weight in g.	133.5±1.3^{c}	132.8±1.4^{c}	134.5±1.4^{c}	147.3±1.7^{a}	142.05±1.92^{b}
Brain weight in g	0.97±0.2^{a}	0.86±0.4^{b}	0.98±0.1^{a}	1.06±0.4^{a}	1.05±0.2^{a}
Brain/Body %	0.72	0.65	0.72	0.72	0.73
Liver weight in g	3.21±0.22^{a}	2.66±0.12^{b}	2.81±0.19^{a}	3.33±0.24^{a}	3.04±0.14^{a}
Liver/Body %	2.40	2.00	2.09	2.62	2.14
Kidney weight in g	0.78±0.04^{a}	0.62±0.03^{b}	0.78±0.01^{a}	0.81±0.02^{a}	0.80±0.01^{a}
Kidney/Body %	0.58	0.47	0.58	0.55	0.56

Date are represented as mean ± SEM (standard error of the mean) and category letter

According to body weight, it can be divided into three groups with significant difference among the three groups.

First group contained control treatment (control+) with 147.3 g. The second group, contained control treatment (control-) with 142.05 g. and the third group contained salicylic acid, Bio Arc and Bio Zeid with 133.5, 132.8 and 134.5 g respectively. Mean brain weight of rats fed on fodder mixed with tomato juice ranged from 0.86 g. for rats fed on fodder mixed with tomato juice treated

with Bio Zeid to 1.06 g. for rats fed on fodder mixed with tomato juice from untreated plant (control+). Only Bio Zeid resulted in decreased brain weight compared with all the other treatments. No significant difference was found among any of the other treatments. Calculating the relative brain weight to body weight revealed the relative brain of rats fed on fodder mixed with tomato juice treated with Bio Zeid represented only 0.65% of the body weight; while relative brain weights in all other treatments represented 0.72 to 0.73%of the body weight. Similar to brain, liver mean weight ranged from 2.66 g, for rats fed on fodder mixed with tomato juice treated with Bio Zeid to 3.33 g. for rats fed on fodder mixed with tomato juice from untreated plant. Bio Zeid and bio Arc resulted in significant decreased liver weight (2.66 and 2.8g) respectively, compared with all the other treatments, salicylic acid with 3.21, control+ with 33.3g and control-with 3.04g.Without any significant difference among any of them. At the same time other rats fed on fodder mixed with tomato juice treated with Bio Zeid showed the least relative liver weight (2.00%); followed by Rats fed on fodder mixed with tomato juice treated with Bio Arc with 2.09% then rats that received only fodder with 2.14. Rats fed on fodder mixed with tomato juice, without any other treatment, resulted in relative liver weight of 2.62%, followed by rats fed on fodder mixed with tomato juice treated with salicylic acid with 2.40%. According kidney weight, rats fed on fodder mixed with tomato juice treated with BioZeid recorded the least kidney weight (0.62g), with significant difference between this treatment on one side, and all the other treatments on the other side. Calculating relative kidney weight to body revealed that, kidney weight of rats fed on fodder, mixed with tomato juice treated with BioZeid, represented only 0.47% of the body compared with 0.58%, 0.58%, 0.55% and 0.56% for rats fed on fodder mixed with tomato juice treated with salicylic acid, Bio Arc and the two control treatments respectively.

4. DISCUSSION

Pesticides have a specific purpose in society; they are intended to control insects, fungi, bacteria and weeds that damage crops, however, pesticides can adversely affect some nontarget living organism. Even the least-toxic products, and those that are natural or organic products, can cause human health problems; specially, if someone is exposed to enough amounts of their residues (Anonymous, 2015), therefore it becomes inevitable to develop a bio-based, eco-friendly, biodegradable plant derived pesticides or microbial pesticides, in order to control plant pathogens. Biological control or bio-control using antagonistic microorganisms, resistance inducers and growth promoters offer a practical and economical alternative for the management of plant pathogens (Chandrashekara, *et. al*., 2012). In this Study the impact of Bio Arc (*Bacillus megaterium*), salicylic acid and humic acid on *Alternaria solani* the causal organism of tomato early blight disease were assessed and compared with the recommended biocide BioZied (*Trichoderma album*).In *vivo*, Bio Arc was the most effective one followed by Bio Zeid then salicylic acid, in contrast to humic acid, which resulted in very low efficacy. On the other side, while disease severity on plants treated with Bio Zeid increased gradually tell after the third spray, disease severity on plants treated with Bio Arc and salicylic acid increased gradually tell the second spray, then began to decrease. This may due to that Bio Zeid (*Trichoderma album*) acts with only one mode of action, depend upon antagonistic effect only on the pathogen, by production of cyclopentenone derivative metabolite (George *et. al*., 1977 and Strunz *et. al*, 1977); while Bio Arc (*Bacillus megaterium*) acts with more than one mode of action (Kildea, *et. al.*, 2008).*Bacillus megaterium* antagonists the pathogens, as a result of production of metabolite "megacin" which causes a radical change in the osmotic barrier of sensitive organisms by attacking the cytoplasmic membrane (Ivfiovics *et. al*, 1959 and Khalil *et.al*, 2009). In addition, it act as resistance inducer, since it leads to an increase in polyphenolics; as well as in defense related enzymes-peroxidase, chitinase, β-1,3-glucanase, and phenyl alanine ammonia lyase. On the other hand, it acts as growth promoter, since it is able to solubilize phosphate, produce indole acetic acid (IAA), and siderophore (Chakraborty, *et. al*.,2006).As well as, salicylic acid demonstrates many properties, it has most widely consumed in medicine as analgesic, antipyretic, and anti-inflammatory agent, in addition to its keratolytic, bacteriostatic, fungicidal, and photo protective properties (Madan and Levitt, 2014). Lately, salicylic acid is used in agriculture as alternative to fungicide, as resistant inducer (Wang, *et al*., 2007) and growth promoter (Sharma, 2013). Treating tomato plants with Salicylic acid 100 mM led to significant increase in plant height as well as number of branches/plant (El-Mohamedy, *et.al*., 2014).The Plant resistance induction (PRI), side by side with growth promoter property of BioArc and salicylic acid may explain, why in this study the disease severity on plants treated with any of them decreased after the second spray. In plant resistance induction (PRI), based on "-omics" studies, it was recently suggested to divide the priming phenomenon into three different stages: a "priming phase", a "post-challenge primed state" and a "transgenerational primed state .In the first stage, which is true for all types of PRI responses the levels of various transcripts, proteins and metabolites are altered, putting the plant in a stand by state. In the post-challenge primed state reactions combating the stress or are induced (Alexandersson, *et. al* 2016), which indicates that, in this study the post-challenge primed state was fully intact after the second spray .In addition, plant growth promoters resulting in production of new foliage, which could quickly replace the damaged foliage with new healthy, and thus, the proportion of the foliage diseased area to the healthy area decreased .Since, tomato fruits picked many time in the season, therefore, it was important to refer to the disease severity at the whole fruiting period, specially area under disease progress curve showed that, the real disease control efficiency of Bio Zeid, Bio Arc , Salicylic acid and humic acid were, 27%, 28.5%, 17.3% and 15.4% compared with 43%, 67.8%, 40% and 35.4% respectively. Thus, it was useless to introduce humic acid in further studies. In this study, feeding rats for 4 weeks on fodder mixed with juice of tomato fruits harvested from plants treated with salicylic acid showed no significant adverse effect on ALT, HDL and albumin; in contrast to AST, cholesterol, Triglyceride, LDL, creatinin and urea which were significantly increased. These results are in part in agreement with that was found by (Hossne, *et al*. 2007) when they studied the effects of acetylsalicylic acid solutions on VX2 liver carcinoma in rabbits, they found a progressive increase in AST levels over time, although no statistically significant differences were found. On the other hand, the data were obtained in this study are in contrast to the data obtained by (Figueroa-Pérez, *et. al*, 2015) when they studied

the effect of salicylic acid treatment during cultivation of the peppermint plant on the improvement of the anti-diabetic effects of its infusions, they found that, treated peppermint infusions decreased LDL and increased HDL levels. In addition, all groups treated with peppermint infusions had lower serum and liver triglyceride contents and decreased serum transaminase levels. They referred these results to the increase in steroidal saponins and alkaloids, such as trigonellin in peppermint, with other word it can concluded that the effect of salicylic acid on such peppermint plants may due in part to indirect effect; by affecting some other components of the treated plant, which in turn affect the experiment animal physiology. Since the components of tomato fruits differ from peppermint plant, it is to expect different results. On the other hand, feeding rats for the same period on fodder mixed with juice of tomato fruits harvested from plants treated with Bio Zeid showed no significant adverse effect on AST, cholesterol, HDL or albumin; in contrast to ALT, triglyceride and LDL, creatinin and urea; since their level raised significantly. (EL-Dakar,2015) reported that, the biocides Bio Zeid at the low dose (25% of feed) resulted in lower level of cholesterol in mice blood, she attributed that to the elucidation of lovastatin and its analogs, e.g. simvastatin, rosuvastatin, atorvastatin and pravastatin (members of a drug family generally called "Statins"), they areinhibitors of 3-hydroxy-3-methylglutaryl-CoA reeducates (HMG-CoA reeducates), a rate-limiting enzyme of cholesterol biosynthesis. She added, cholesterol level elevation was recorded in blood of mice fed on higher dose (50%) of strawberries treated with Bio Zeid, in addition to significantly increased transaminases levels, which may due to liver hypertrophy caused by excessive statins (Pandit, *et.al*., 2012 and Grimbert, *et.al*., 1994). At the same time, feeding rats for the same period on fodder mixed with juice of tomato fruits harvested from plants treated with BioArc showed no significant adverse effect on ALT and AST. On the other hand, the levels of cholesterol, triglyceride, LDL, creatinin and urea were significantly elevated. In contrast, HDL and albumin were significantly decreased. creatinin and urea level. BioArc (*Bacillus megaterium*) is a bioagent acts by production of Megacins, and antibiotic (VonTersch and Carlton, 1983).However, Megacins kill the cell by inducing drastic changes in the permeability properties of protoplasts and of whole cells, since the homogeneity and density of the cell cytoplasm gradually decreased until finally, only almost empty envelopes remained (Holland,*et.al.,* 1967), ALT and AST level did not raised; which indicated that the liver was not adversely affected. In contrast cholesterol, triglyceride, and LDL significantly increased and HDL and albumin were significantly decreased, that may due to that, *Bacillus megaterium* is able to oxidize various xenobiotic substrates such as, simvastatin and lovastatin, which are used to treat hyperlipidemia and hypercholesterolemia (Kang, *et al.,* 2009), which explains why the cholesterol, triglyceride and LDL level increased and HDL level decreased. Salicylic acid and Bio Zeid resulted in significant increase of AST and ALT respectively. AST and ALT are enzymes found mainly in the liver, but also in some other organs, their levels in combination with other enzymes are a valuable aid primarily in the diagnosis of liver disease .When, body tissue or an organ, such as the liver or heart, diseased or damaged, additional AST and ALT are released into the bloodstream, causing the levels of the enzyme to rise (Huang, *et al*., 2006); this fact indicates that, increased AST and ALT level in blood of rats fed on fodder mixed with juice of tomato fruits harvested from plants treated with Salicylic acid or Bio Zeid, may due to some liver damage, specially the least liver weight was recorded in rates fed on fodder mixed with juice of tomato fruits harvested from plants treated with Bio Zeid and Bio Arc. Creatinin and urea level in blood are a sign of kidney condition; salicylic acid, Bio Zeid and Bio Arc in fodder resulted in raised level of creatinin and urea level, which indicates that the kidneys in these treatments may be adversely affected especially, in case of Bio Zeid, since significant decreased weight of kidney was found. On the other hand, rats fed on fodder mixed with juice of tomato fruits harvested from plants treated with Bio Zeid showed significant decreased of brain and liver weight. *Trichoderma* species have always been considered as a contaminant in organ site cultures. However, in the recent literature, many *Trichoderma* species has been increasingly reported as etiologicagents inhuman infections (Ariese,*et.al*.2013)*Trichoderma longibrachiatum*,*T. harzianum*, *T. koningii*, *T. pseudokoningii* and *T.viride* infections have been reported in patients undergoing immunosuppressive therapies (chemotherapy for solid tumors, hematological malignancies, and after bone marrow or solid organ transplantation) and in patients with peritoneal dialysis. Among the 23 cases reported in the literature so far, infections included sinusitis, necrotizing stomatitis, and abscesses in pulmonary, brain or liver (Lagrange *et.al*, 2008). On the other hand, Bio Zeid (*Trichoderma album*), acts by production of cyclopentenone derivative metabolite (George,*et.al*., 1977 and Strunz, *et.al*.1977); cyclopentenone IsoPs, are highly reactive electrophiles, these compounds also potently induce neuronal apoptosis, by a mechanism which involves glutathione depletion, ROS generation, and activation of several redox-sensitive pathways, enhancing neuro degeneration in brain (Musiek, *et.al*.,2007). As early as 1978 Aspirin was shown to reduce the glomerular filtration rate in 13 patients with renal impairment (Julian, 1978), later, (Doi and Horie , 2010) recorded that salicylic acid induced hepatotoxicity triggered by oxidative stress, causing serious liver damage. They added, mitochondrial dysfunction and oxidative stress are predicted to be the major factors of salicylic acid-induced liver injury. It was concluded that, Bio Arc (*Bacillus megaterium*)and salicylic acid have good biocidal effect on *A. solani*, the causal agent of tomato early blight disease, compared with Bio Zeid (*Trichoderma album*),the recommended biocide and could be promising tomato early blight disease biocides; it is better to be used as protective before the infection established. However, toxicological studies revealed pronounced toxic risk factor to liver, kidney and brain. Further histopathological studies have to be done to confirm this finding.

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