International Journal of Agricultural Technology 2022Vol. 18(6):2381-2398
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

Microorganisms in biocontrol of plant pathogens: toxic effects on experimental rate

Haggag, W. M.[1*]and Shalaby, Sh. E. M.[2]

[1]Department of Plant Pathology, National Research Centre, Cairo, Egypt; [2]Department of Pests and Plant Protection, National Research Centre, Dokki, Cairo, Egypt.

Haggag, W. M. and Shalab, Sh. E. M. (2022). Microorganisms in biocontrol of plant pathogens: toxic effects on experimental rate. International Journal of Agricultural Technology 18(6):2381-2398.

Abstract Beneficial microorganisms using biocontrol agents to reduce the use of unsafe chemical fungicides, give a potent alternative to control plant diseases and is a significant part of sustainable agriculture. Our previous study of a advantage of using biological control showed that biologically, biochemistry and physiology of production of antifungal substances are well documented. Therefore, the potentiality and toxic effects of some beneficial microorganisms included *Pseudomonas putida* , *Streptomyces aureofaciens* , *Rhodatorula glutinis***,** *Trichoderma harzianum* as well as Algae, *Oscillatoria geminate* on experimental rate were done to combat plant pathogens. The antifungal activities of microorganisms were tested against the five tested foliar pathogenic organism's i.e. *Fusarium oxysporum*, *Pyrenophora teres*, *Septoria tritici*, *Botrytis cinerea* and *Alternaria solani*. The results showed that *S. aureofaciens*, *P. putida* and *R. glutinis* had effective against foliar pathogens. In addition, antifungal metabolites of the cell free bacterial and fungal culture media caused a significant reduction in all pathogen's growth and germination. Hematological effective of synthetic and biological fungicides showed that the used chemical fungicide for comparison caused effects at recommendation rate. All biocontrol agents has a high degree of safety and the highest biosafety degree which was shown in *S. aureofaciens*, *P. putida* and *R. glutinis* which compared to chemical fungicide. It is recommended that can be using the tested bio-agents as alternative chemical fungicides.

Keywords: Biocontrol, Toxic Effects of biofungicides, Hematological, Experimental rate

Introduction

The pollution effects of pesticides on the environment play a serious role in the incident of numerous diseases affecting living organisms. Biological control offers an environmentally friendly alternative to the use of pesticides for controlling plant diseases in field and postharvest (Haggag and and Abouziena, 2016 and Betsabee *et al*., 2017). Various antagonistic agents produce secondary metabolites which can suppress other microorganisms.

Biological control offers an environmentally friendly and safe alternative to the use of pesticides for controlling plant diseases (Haggag and Abouziena, 2016 and Haggag and Ali, 2019). Several microorganisms

[*] **Corrsponding Author:** Haggag W. M.; **Email**:Wafaa_haggag@yahoo.com

as *Streptomyces* spp. *and Pseudomonas putida* produce broad spectrum natural metabolites as antibiotics and lytic enzymes, which can degrade cell wall of other organisms that make them advantageous for plant disease biocontrol against anthracnose, die back and grey mould (Madbouly, 2018). *Streptomyces* spp. is shown to be promising biocontrol agents against a wide range of plant pathogenic fungi that produce different bioactive compounds (Fei Law *et al*., 2017). In addition, Duke *et al.* (2017) stated that *Pseudomonas* spp. can colonize the host plants with induction of defense against diseases. Haggag and Abouziena (2016) found that *Rhodotorula glutinis* showed antioxidant and antifungal trends against blight and spots causing fungal pathogens e.g. *Aspergillus flavus, A. niger, Alternaria triticina, Fusarium moniliforme* and *Penicillium chrysogenum* on wheat grain and significantly enhanced plant growth and yield. Algae (cyanobacteria) are a diverse group of organisms that frequently occur in marine environment and play a role in soil fertility, reclamation, protect plants from pathogens, change of microbiological system and can stimulate plant growth that show a great potential for generation of novel agricultural technologies derived benefit and as a source of compounds act as biofungicides products (Haggag *et al*., 2015). In addition, as studies and research on biocontrol agents have beed moved forward, there have also been reported about their toxicity as well as the toxicity of purified metabolites (Shalaby and Abdou, 2020). Therefore, considering the increase in the global market demand for natural products that meet consumer demands for health and well-being, it is clearly known that there is a promising future for the production of biopesticide compounds through toxicity using microorganisms, especially if we take into account the disadvantages of natural products obtained from plants with high extraction and purification costs and a low metabolites yield, commonly affected by environmental conditions.

Materials and methods

Biocontrol agents

Biocontrol agents are previously isolated from Egyptian soil and tested against phytopathogenic fungi were used in this study i.e *Pseudomonas putida , Streptomyces aureofaciens, Rhodatorula glutinis, Trichoderma harzianum* as well as Algae, *Oscillatoria geminate* (Haggag, and Ali, 2019 , Haggag and Abouziena, 2016 , Haggag *et al.,* 2015). Microorganisms were identified in Plant Pathology Department, National Research Centre, Egypt. Culture was grown and maintained on solid starch medium (Kuster and Williams, 1964) at 28℃, nutrient agar and potato dextrose agar (PDA) media respectively. The isolates were incubated in a rotary shaker (200 rpm) for 72 h at 25 ±3 ℃. Cells were harvested by

centrifugation at 6,000 rpm for 10 min, washed twice with sterilized water and re-suspended in sterilized distilled water. The concentrations of bacteria cells in the suspensions were adjusted to $1x10^6$ cells / ml and $1x10^4$ cells / ml (cfu / mL) for fungi.

Antifungal properties of microorganisms

Microorganisms were tested against the plant pathogens e.g. *Fusarium oxysporum*, *Pyrenophora teres*, *Septoria tritici*, *Botrytis cinere* and *Alternaria solani*. Both the inhibition of hyphae growth and the germination of spores of pathogens were determined. Inhibition was determined as zone of inhibition. The antimicrobial activities of the cell free culture media of both soil bacteria and fungi on the growth of the tested pathogenic microbes were tested using the method described by Umechuruba and Nwachukwa (1997) .

Spore germination test

Spores of fungal pathogens were adjusted to a final concentration of 5×10^5 spores ml^{-1}, placed onto sterilized glass microscope slides and incubated at 25 ℃ and 100% RH. After pre-incubation at 250 mins, a 25 µl aliquot of cell free culture of different bioagents at 50% concentration which were added to each droplet and mixed gently with a pipette tip. Control samples were also prepared in which the cell-free culture was replaced with 25 µl sterile media. The percentage of germination was determined using ten microscope slides per treatment.

Biosafety and toxicity studies

The tested compounds were used as Dithane M 45 % WP was used as control. It tested against *Oscillatoria geminate, Streptomyces aureofaciens, Pseudomonas putida, Rhodatorula glutinis* and *Trichoderma harzianum.*

Tested animals

Female albino rats *Rattus norvegicus* var. albinos (weighing approximately 110 – 120 g) which used in this study were obtained from the Animal House, National Research Centre (NRC), Cairo, Egypt. Seven days before starting the experiment, the animals were housed in plastic cages and allowed to set to the suitable environment and fed standard food pellets and tap water *ad libitum*. The rats were housed at 25 ± 2 ℃ and daily dark / light cycle. The design of the study was in accordance with the ethical guidance prescribed by NRC. Rats were randomly divided into seven groups and separately caged and treated day after two days during one month. The

first group was used as controls. The second to six groups were orally administered with 100 mg (formulation) /rat of *O. geminate, S. aureofaciens, P. putida, R. glutinis* and *T. harzianum* bio-compounds. The 7^{th} group was treated with 1/10 of the LD_{50} of Dithane fungicide (5000 mg / kg according to Anonymous, 2012). This was carried out by catching the tail, holding its back and stretching the skin to force open its mouth. Toxicants were dissolved in corn oil and administered by stomach tube. After 15 and 30 days from treatment, treated animals were sacrificed, blood samples were collected for measuring the chosen parameters. All animals were observed daily for signs of pharmacological or toxicological effects.

Hematological tests

Blood samples were collected in anticoagulant tubes for hematological parameters; RBCs, WBCs counts and hemoglobin values were determined according to Schalm (1986) method.

Biochemical analysis

At 15 and 30 days recovery periods, blood samples were taken from retro-orbital venous plexus, placed into sterile tubes and centrifuged at 3500 rpm for 20 min to separate the serum.

Biochemical parameters

The activity of blood serum transaminases [aspartate transaminase (AST) and alanine transaminase (ALT)], urea and creatinine were determined, and analyzed spectrophotmetrically using kits purchased from Bio-Marieux Company France. Using automated clinical chemistry analyzed Olympus Au 4oo Analyzer. The experimental design was a factorial in Complete Randomized Design (CRD) with ten replicates. Statistical analysis of the collected data was carried out using a computer program (Cohort Software, 1986), and treatments compared with Duncan′s Multiple Range Test.

Statistical analysis

The efficiency of all trials was analyzed with ANOVA and means was compared with the least significance difference (LSD) test using SAS statistics. Pair wise comparison of isolates, based on the presence (1) or absence (0) of unique and shared polymorphic products was used to generate similarity coefficients using statistical software package STATISTICA-SPS. The different statistical analysis such as correlation, regression, LSD etc. were applied to reach final descison of the suitability of clean farming on different crops.

Results

Microorganisms and bioassay

The antifungal activities of microorganisms were tested against the five tested foliar pathogenic organisms' i.e. *F. oxysporum*, *P. teres*, *S. tritici*, *B. cinere* and *A. solani*. The results showed that *S. aureofaciens*, *P. putida* and, *R. glutinis* had the highest effect as antifungal activity against foliar pathogens compared to the other microorganism (Table 1 and Figure 1). The same trend was observed for the cell free bacterial and fungal culture media against *F. oxysporum*, *P. teres*, *S. tritici*, *B. cinere* and *A. solani*. Thus, the pathogenic organisms showed higher sensitivity to the filtrates resulted from *S. aureofaciens* and *P. putida* (Table 2 and Figure 2). Antifungal metabolites of the cell free bacterial and fungal culture media caused a significant reduction (*P*=0 05) in all pathogen's germination compared with untreated controls when used at 50% conc (Table 3 and Figure 3). The treatments showed that *S. aureofaciens* metabolites were involved. However, *P. putida* gave higher effect on reduction of spores' germination.

Biosafety and toxicity studies

Effects on body weight of tested rats showed that revealed that there was a significant difference in body weight in rats treated by dithane compared with other treatments (Table 4). Dithane fungicide caused to decrease in rat's body weight after 30 days of treatment and after recovery period, while there were negligible effects between bio-compounds and untreated rats during all intervals of experiment. Also, it can be arranged the average of body weight treatments in descending (Figure 4) as *R. glutinis* (187.5g) and followed by untreated rats (185.0g), *Trichoderma, Pseudomonas, O. geminate* (181.5 g), *Streptomyces* (177.0 g) and dithane (175.0 g).

Hematological effects revealed significantly decreased in hemoglobin values (Hb) in treated rats by chemical fungicide (dithane) after 30 days compared by other bio-materials and untreated animals without return to normal level after recovery period (15 days) as seen in Table 6. In the same respect, there was not significantly changed in Hb values in bio-materials treated rats between each other and compared with untreated rats (Table 5 and Figure 5). On the other hand, after 15 days for treatment dithane caused significantly increasing in white blood cells (WBCs) when compared with other bio-agents except in case of *Streptomyces* treated rats (4.2 X 10^3 /ml), while no significant changeg in all treatments compared with control except in *Pseudomonas* treated rats (3.4 X 10^3 /ml) after 30 days for application. At the end of experiment (recovery period), there was not significant changed in WBC counts in all treatments with exception in dithane treated rats (5.1 X 10^3 /ml) (Table 6 and Figure 6). On the contrary, dithane, *Pesudomonas* and *R. glutinis* treatments were significantly decreased in red blood cell counts (RBCs) after 15 days of treatment

compared with other bio-agent and untreated rats (Table 7 and Figure 7). These effects of RBCs decreasing that was noticed after 30 days in rats treated by dithane (4.6 X 10^6/ml), *R. glutinis* (5.1 X 10^6/ml) and *Trichoderma* (5.1 X 10^6/ml). After the recovery period, there was not significantly changed which observed in RBCs counts in all treated rats.

Effects on liver functions revealed that the chemical fungicide dithane and tested bio-agent were significantly increased in alanine transaminase (ALT) enzyme activity in treated rats compared with control, except in case of *R. glutinis* treated rats after 15 days for application (Table 8 and Figure 8). More effects noticed after 30 days in case of dithane treated rats while no significant changeswas happened in bio-agent treatment compared with untreated rats. After recovery period, ALT activity was still significantly increased in dithane and algae treated rats, but returned to its normal level in other treatments compared with control. In same trend, It revealed that dithane fungicide was significantly increased in aspatate transaminase enzyme (AST) activity in treated rats compared with bio-agent materials and untreated rats, while there were not significantly changed in the enzyme activity in treated rats by bio-agents compared with control group (Table 9 & Fig.9). These effects were observed during all experiment periods and did not return to normal level in dithane treated rats after recovery period. Data indicated that dithane had the highest average of AST activity (36.8 mg/dl), followed by Streptomyces and *Oscillatoria* (28.9 and 28.47 mg/dl), then untreated rats (27.2), *R. glutinis*, Pseudomonas and *Trichoderma* (26.59, 26.57 and 26.23 mg/dl) respectively.

Effects on kidney functions

Results showed there are no significant changes in creatinine concentrations after 15 days for application in all treatments compared with check groups as presented in Table 10 and Fig. 10. After 30 days dithane chemical fungicide caused significant increase in creatinine amount (1.08 mg/dl) compared with other treatments and control. At the end of recovery period, creatinine concentrations return to normal level approximately in all treatments. On the anther side, there were not significantly changed which noticed in urea concentration in all treated rats compared with control after 15 days for treatment (Table 11); while Pseudomonas treated rats had the lowest urea amount (29.8 mg/dl). This trend was noticed after 30 days, but there was significantly increased in urea amount in dithane treated rats compared with bio-agent treated rats, and *T. harzianum* treated rats had the lowest urea concentration (31.5 mg/dl). At the end of recovery period significantly increase in urea amount was noticed in rats treated by dithane, *Oscillatoria*, *Streptomyces* and *Pseudomonas* compared with check group. Also, dithane treated rats had the highest urea concentration (34.97 mg/dl), while *T. harzianum* treated rats had the lowest amount (31.7 mg/dl) (Figure 11).

Table 1. The antagonistic activity (expressed by the inhibition zone) of microorganisms on some foliar pathogenic fungi

Treatments	Zone of inhibition (mm)						
	Fusarium oxysporum	*Pyrenophora teres*	*Septoria tritici*	*Botrytis cinerea*	*Alternaria solai*	*Lasiodiplodia theobromae*	*Colletotrichum gloeosporioides*
Oscillatoria geminate	8.8	11.9	12.6	9.6	8.4	6.8	7.4
Streptomyces aureofaciens	17.7	16.7	15.8	14.8	15.9	17.8	16.7
Pseudomonas putida	12.7	11.7	14.7	13.3	14.7	16.6	16.3
Rhodatorula glutinis	14.8	15.7	13.7	14.0	15.6	9.7	13.6
Trichoderma harzianum	11.5	12.7	11.5	12.8	11.8	12.7	11.7
LSD 5%	1.3	1.4	1.5	1.1	1.0	0.86	0.88

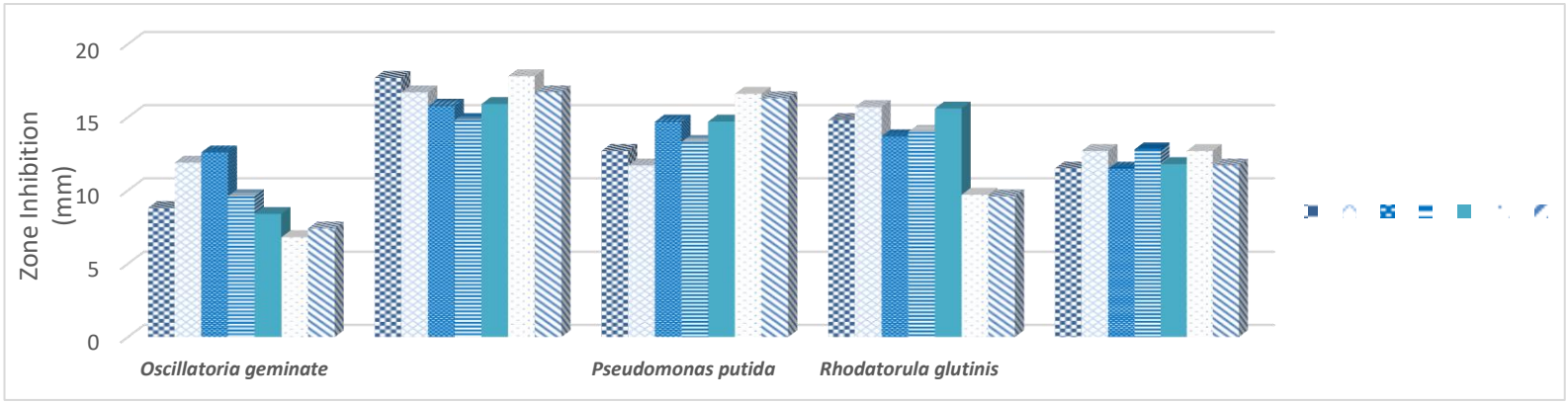

Figure 1. The antagonistic activity (expressed by the inhibition zone) of microorganisms on some foliar pathogenic fungi

Table 2. The antagonistic effect of microorganisms cell free culture medium on the growth of *some foliar plant pathogens*

Treatments	*Zone* inhibition *(mm)*						
	Fusarium oxysporum	*Pyrenophora teres*	*Septoria tritici*	*Botrytis cinerea*	*Alternaria solai*	*Lasiodiplodia theobromae*	*Colletotrichum gloeosporioides*
Oscillatoria geminate	9.1	9.8	11.7	5.7	5.4	5.1	5.2
Streptomyces griseus	18.8	16.7	14.8	17.8	14.5	18.6	18.7
Pseudomonas putida	13.7	12.2	14.7	12.3	12.7	15.7	15.6
Rhodatorula glutinis	13.8	15.5	13.6	14.7	14.6	11.7	10.7
Trichoderma harzianum	11.5	12.7	11.5	12.8	11.8	12.7	12.8
LSD 5%	1.3	1.35	1.5	1.1	1.0	1.1	1.1

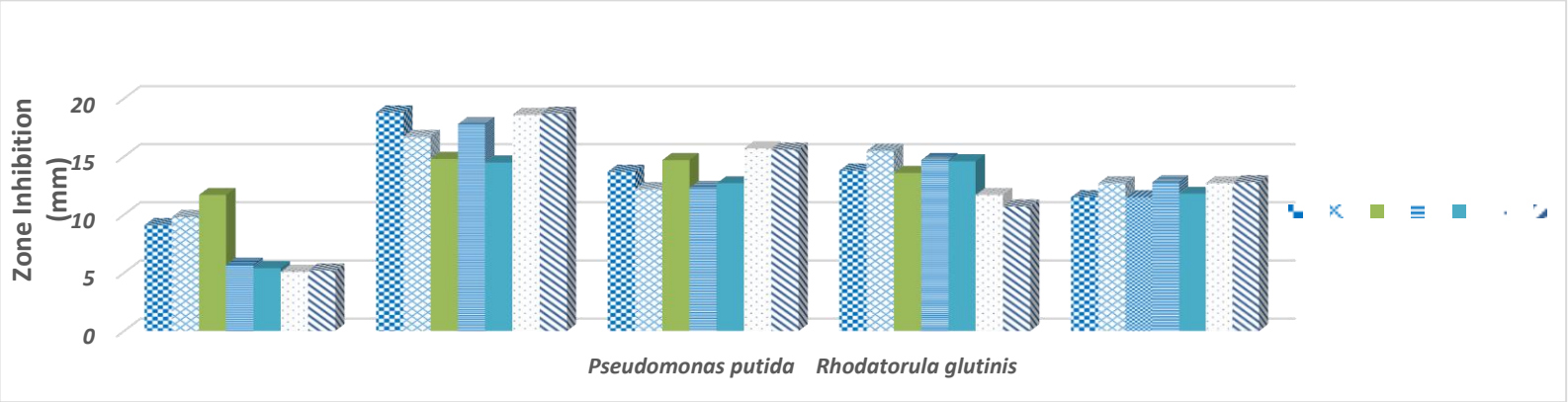

Figure 2. The antagonistic effect of microorganismsl cell free culture medium on the growth of some foliar plant pathogens

Table 3. The antagonistic effect of microorganisms cell free culture medium on the inhibits germination *of some foliar plant pathogens*

Treatments	*Spores Germination %*						
	Fusarium oxysporum	*Pyrenophora teres*	*Septoria tritici*	*Botrytis cinerea*	*Alternaria solai*	*Lasiodiplodia theobromae*	*Colletotrichum gloeosporioides*
Oscillatoria geminate	25.8	35.7	24.8	23.8	25.7	29.7	23.8
Streptomyces griseus	1.8	5.5	4.8	2.7	3.6	3.7	3.5
Pseudomonas putida	2.2	6.6	6.8	4.7	5.8	4.8	5.8
Rhodatorula glutinis	8.8	9.8	9.6	10.7	11.0	9.8	10.8
Trichoderma harzianum	7.8	9.7	9.8	7.8	8.9	9.8	9.9
Control	85.7	75.8	77.8	95.6	85.7	74.8	78.8
LSD 5%	1.2	1.4	1.7	1.8	1.9	1.8	1.7

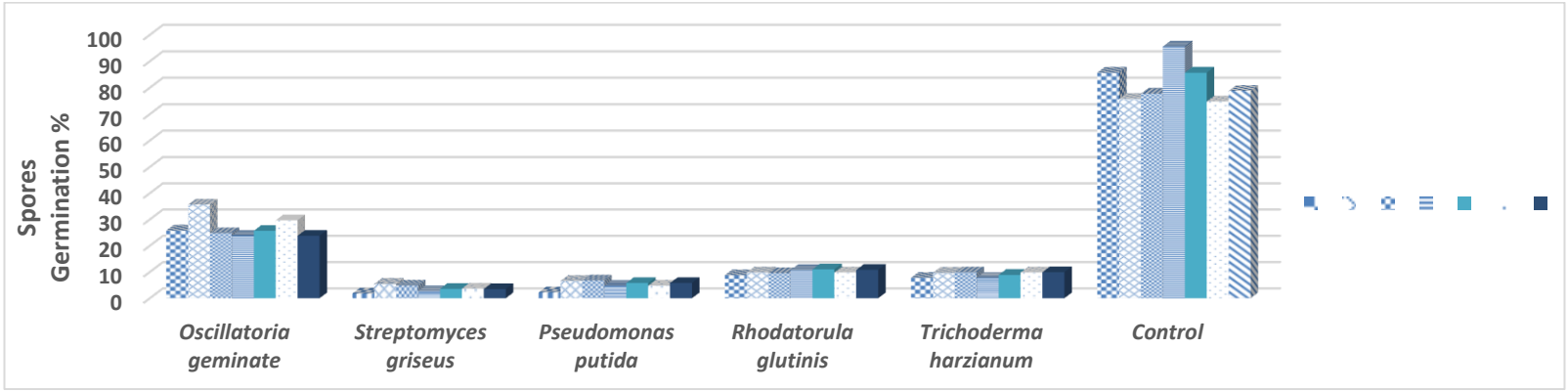

Figure 3. The antagonistic effect of microorganisms cell free culture medium on the inhibits germination of some foliar plant pathogens

Table 4. Effect of tested bio-fungicides on body weight of white albino rats

Periods **Compound**	**Pre-treatment (g)**	**15 days (g)**	**30 days (g)**	**15 days for recovery (g)**	**Average**
Dithane	152 a	172 ab	182 d	194 b	175.0
Oscillatoria geminate	148 a	166 bc	196 bc	214 a	181.0
Streptomyces griseus	150 a	162 c	188 cd	208 a	177.0
Pseudomonas putida	152 a	168 bc	194 bc	212 a	181.5
Rhodatorula glutinis	150 a	178 a	208 a	214 a	187.5
Trichoderma harzianum	150 a	166 bc	192 bcd	218 a	181.5
Control	148 a	176 a	196 bc	220 a	185.0
LSD 5%	11.25	8.6	10.4	12.5	

(According to Duncan test) Letters means the significant differences between treatments. Each figure between brackets represents the percentage of content as check

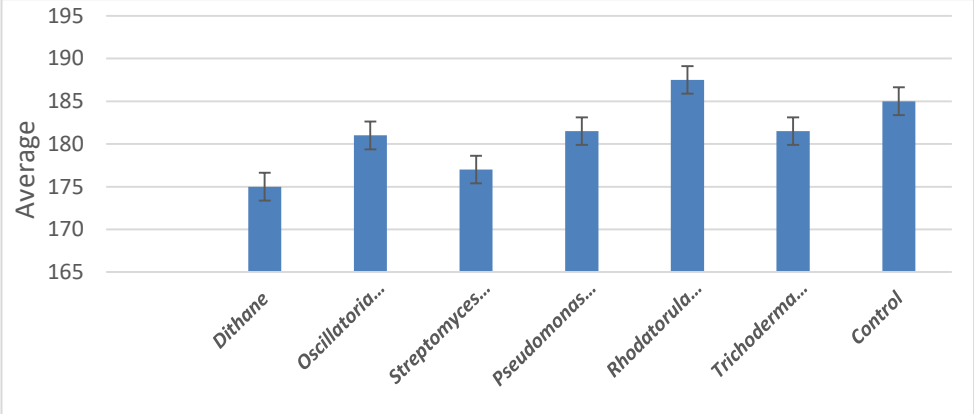

Figure 4. The average of treated rat's body weight (g)

Table 5. Effect of tested bio-fungicides on hemoglobin values of white albino rats

Periods Compound	15 days (mg/ml)	30 days (mg/ml)	15 days for recovery (mg/ml)	Average (mg/ml)
Dithane	11.9 ab	10.8 c	10.7 c	11.13
Oscillatoria geminate	11.5 c	12.1 a	11.4 b	11.66
Streptomyces griseus	12.4 a	11.9 a	12.1 a	12.13
Pseudomonas putida	11.7 bc	11.4 b	12.3 a	11.8
Rhodatorula glutinis	12.3 ab	11.8 a	11.7 b	11.93
Trichoderma harzianum	12.1 ab	11.4 b	12.0 ab	11.83
Control	12.2 abc	11.8 a	11.9 ab	11.97
LSD 5%	0.76	0.4	0.54	

(According to Duncan test) Letters means the significant differences between treatments. Each figure between brackets represents the percentage of content as check.

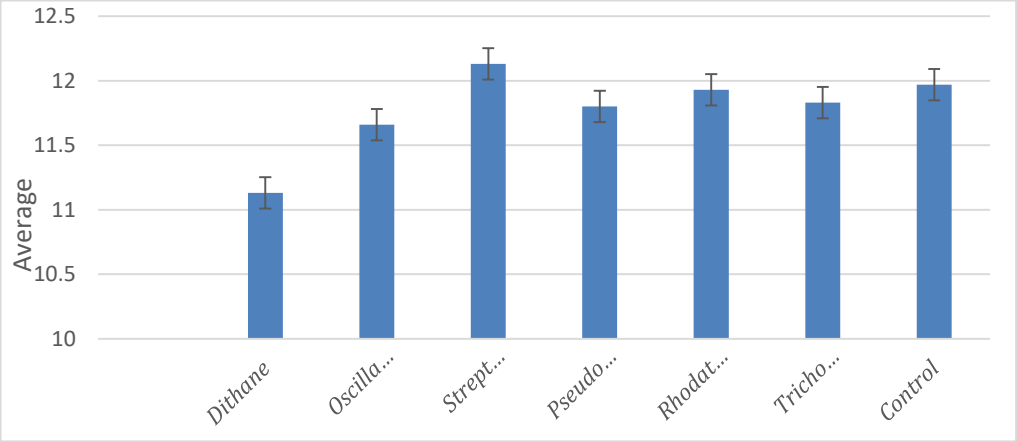

Figure 5. The average of hemoglobin concentrations (mg/ml) of treated rats

Table 6. Effect of tested bio-fungicides on white blood cell counts of white albino rats

Periods **Compound**	**15 days (10^3/ml)**	**30 days (10^3/ml)**	**15 days for recovery (10^3/ml)**	**Average (10^3/ml)**
Dithane	4.7 a	4.6 a	5.1 a	4.8
Oscillatoria geminate	3.8 b	4.2 ab	4.0 b	4.0
Streptomyces griseus	4.2 ab	4.4 ab	4.0 b	4.2
Pseudomonas putida	3.7 b	3.4 c	3.8 b	3.67
Rhodatorula glutinis	3.6 b	4.0 b	3.9 b	3.83
Trichoderma harzianum	4.0 b	4.1 ab	4.1 b	4.07
Control	3.8 b	4.1 ab	4.2 b	4.03
LSD 5%	0.62	0.53	0.70	

(According to Duncan test) Letters means the significant differences between treatments. Each figure between brackets represents the percentage of content as check.

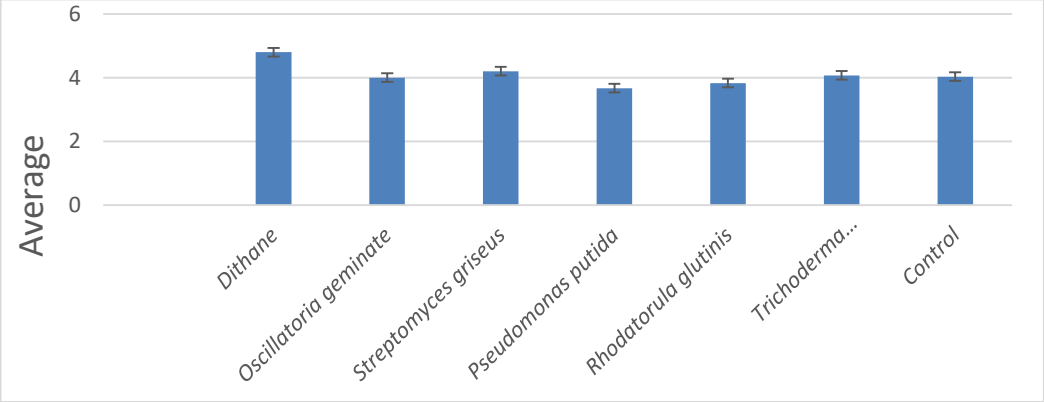

Figure 6. The average of white blood cell counts (WBCs) (10^3/ml) of treated rats

Table 7. Effect of tested bio-fungicides on red blood cell counts of white albino rats

Periods Compound	15 days (10^6/ml)	30 days (10^6/ml)	15 days for recovery (10^6/ml)	Average (10^6/ml)
Dithane	4.8 b	4.6 c	5.1 a	4.83
Oscillatoria geminate	5.3 ab	5.2 abc	5.4 a	5.3
Streptomyces griseus	6.0 a	5.8 a	5.4 a	5.73
Pseudomonas putida	5.2 b	5.4 ab	5.3 a	5.37
Rhodatorula glutinis	5.0 b	4.8 c	5.2 a	5.0
Trichoderma harzianum	6.0 a	5.1 b	5.6 a	5.57
Control	6.0 a	5.8 a	5.5 a	5.77
LSD 5%	0.74	0.65	0.63	

(According to Duncan test) Letters means the significant differences between treatments. Each figure between brackets represents the percentage of content as check.

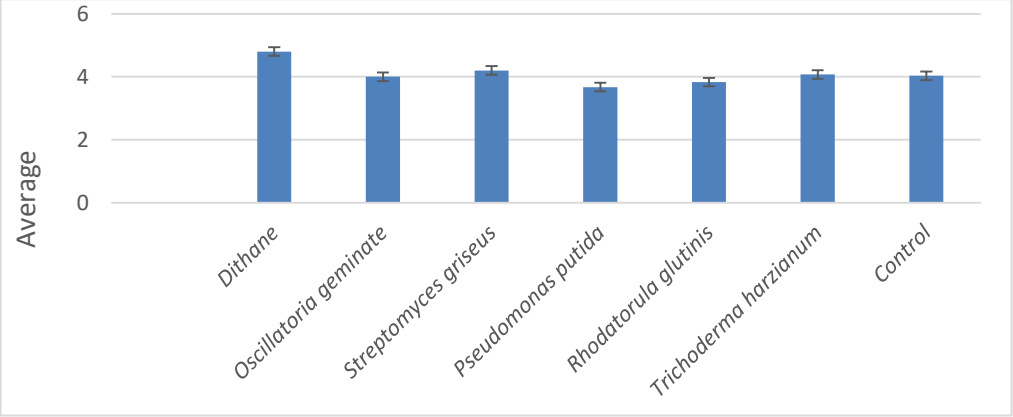

Figure 7. The average of red blood cell counts (RBCs) (10^6/ml) of treated rats

Table 8. Effect of tested bio-fungicides on ALT activity of white albino rat

Periods Compound	15 days (mg/dl)	30 days (mg/dl)	15 days for recovery (mg/dl)	Average (mg/dl)
Dithane	40.4 a	44.5 a	39.8 a	41.57
Oscillatoria geminate	37.2 b	39.4 b	36.8 b	37.8
Streptomyces griseus	37.5 b	34.6 c	35.9 bc	36.0
Pseudomonas putida	39.8 ab	40.0 b	35.9 bc	38.57
Rhodatorula glutinis	36.7 c	38.9 b	38.7 ab	38.1
Trichoderma harzianum	33.9 d	40.0 b	36.1 bc	36.67
Control	36.0 c	37.5 bc	34.8 c	36.1
LSD 5%	2.6	3.1	2.2	

(According to Duncan test) Letters means the significant differences between treatments. Each figure between brackets represents the percentage of content as check.

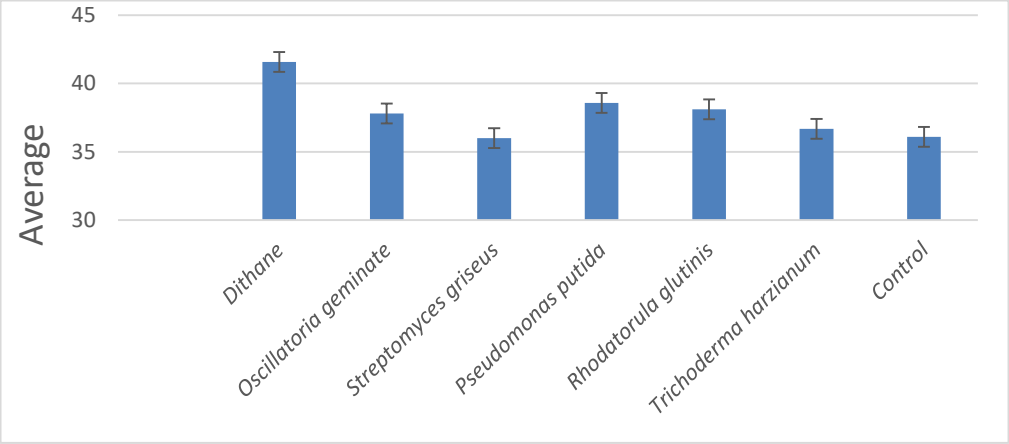

Figure 8. The average of alanine transaminase enzyme activity (ALT) (mg/dl) of treated rats

Table 9. Effect of tested bio-fungicides on AST activity of white albino rats

Periods Compound	**15 days (mg/dl)**	**30 days (mg/dl)**	**15 days for recovery (mg/dl)**	**Average (mg/dl)**
Dithane	35.6 a	38.9 a	35.9 a	36.8
Oscillatoria geminate	28.9 b	30.1 b	26.4 b	28.47
Streptomyces griseus	30.2 b	29.8 b	26.7 b	28.9
Pseudomonas putida	25.7 c	28.9 b	24.9 b	26.57
Rhodatorula glutinis	28.9 b	26.4 b	24.47 b	26.59
Trichoderma harzianum	25.6 c	27.3 b	25.8 b	26.23
Control	27.3 bc	28.6 b	25.7 b	27.2
LSD 5%	3.1	3.7	3.2	

(According to Duncan test) Letters means the significant differences between treatments. Each figure between brackets represents the percentage of content as check.

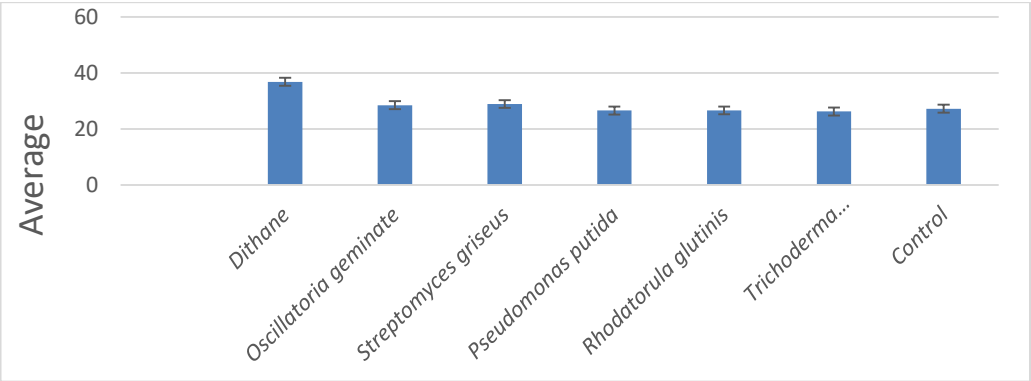

Figure 9. The average of aspartate transaminase enzyme activity (AST) (mg/dl) of treated rats

Table 10. Effect of tested bio-fungicides on creatinine concentration of white albino rats

Periods Compound	15 days (mg/dl)	30 days (mg/dl)	15 days for recovery (mg/dl)	Average (mg/dl)
Dithane	0.92 a	1.08 a	0.9 a	0.97
Oscillatoria geminate	0.77 b	0.85 b	0.87 a	0.83
Streptomyces griseus	0.82 ab	0.79 b	0.9 a	0.84
Pseudomonas putida	0.87 ab	0.79 b	0.86 a	0.84
Rhodatorula glutinis	0.82 ab	0.76 b	0.91 a	0.83
Trichoderma harzianum	0.78 b	0.82 b	0.83 a	0.81
Control	0.85 ab	0.79 b	0.92 a	0.85
LSD 5%	0.12	0.13	0.11	

(According to Duncan test) Letters means the significant differences between treatments. Each figure between brackets represents the percentage of content as check.

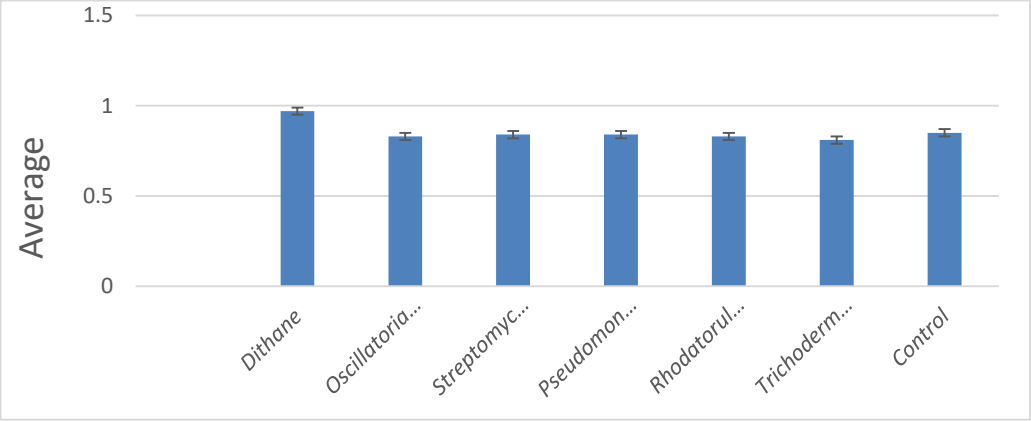

Figure 10. The average of creatinine concentrations (mg/dl) of treated rats

Table 11. Effect of tested bio-fungicides on urea concentration of white albino rats

Periods Compound	15 days (mg/dl)	30 days (mg/dl)	15 days for recovery (mg/dl)	Average (mg/dl)
Dithane	34.2 a	36.0 a	34.7 a	34.97
Oscillatoria geminate	33.6 ab	35.4 ab	35.4 a	34.8
Streptomyces griseus	30.9 bc	32.7 bc	33.4 a	32.33
Pseudomonas putida	29.8 c	32.6 bc	33.7 a	32.0
Rhodatorula glutinis	35.0 a	31.6 bc	32.4 b	33.0
Trichoderma harzianum	30.5 bc	31.5 c	33.1 ab	31.7
Control	32.0 abc	34.6 ab	30.8 b	32.47
LSD 5%	3.1	2.9	2.6	

(According to Duncan test) Letters means the significant differences between treatments. Each figure between brackets represents the percentage of content as check.

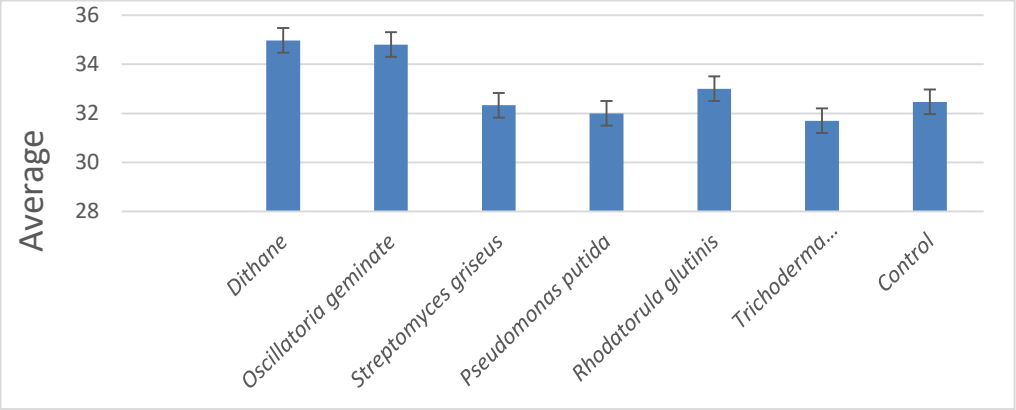

Figure 11. The average of urea concentrations (mg/dl) of treated rats

Discussion

Several microorganisms produce antimicrobial and antifungal components as lytic enzymes, which can degrade cell wall of other organisms. The results showed that most of tested microorganisms gave highest effect as antimicrobial activity against foliar pathogens. From these microorganisms, *S. aureofaciens* known a potential biocontrol agents against several plant pathogens with antibacterial, antifungal, antiviral and other properties due to antibiotic producing microorganism and have against foliar pathogenic fungi (Haggag *et al*., 2015 Haggag *et al*., 2015). It is known to produce a number of bioactive molecules, including the antibiotic streptomycin, and analysis of the complete genome of *S. griseus* suggested that this strain has the capacity to produce over 34 secondary metabolites (Ohnishi *et al.*, 2008). *R. glutinis* and *T. harzianum* and *Pseudomonas putida* are exhibited strong inhibitory activity against plant pathogenic fungi *in vitro and vivo* (Xiao *et al.*, 2013 and Madbouly, 2018). Algae as *Oscillatoria geminate* are a widely organisms in marine environment and play a role in plants protection from pathogens and as a source of compounds act as biofungicides products (Haggag *et al*., 2015).

Hematological effective of synthetic of biocides has been studied on experimental rate. Our results indicated that all biofungicides has a high degree of safety in compared with fungicide. Body weight treatments in descending with *R. glutinis* followed by untreated rats, *Trichoderma*, *Pseudomonas*, *O. geminate* then *Streptomyces* and dithane. The results of hematological effective of biological fungicides on experimental rate are in accordance with those obtained by Adjrah *et al*., (2013) who reported that the decrease in rat body weight was noted and animals have soft feces in rats administered with mancozeb-treated lettuce. The high increase of WBCs may be due to the

inflammatory response induced as defense mechanism. Also, chemical pesticides may affect the WBCs count by the stressogenic effect of these compounds on the reticuloendothelial system (Gromysz, 1993). These unwise changes may be due to the metabolic fates of the tested insecticides and interference of their metabolites with vital compounds of the cells (Shalaby and Abdou, 2020).

Our results of liver functions in accordance with those obtained by Adjrah *et al*. (2013) who reported that plasmatic concentrations of transaminases, alkaline phosphatase, and total bilirubin are increased in rats administered with mancozeb-treated lettuce. Also, data obtained by Yahia *et al*. (2014) showed that mancozeb exposure caused a significant increase in aspartic aminotransferase (AST) and alanine aminotransferase (ALT) in treated rats. The obtained observations clearly reveal hepatotoxic effects of mancozeb in rats and constitute, therefore, an environmental health risks to living organisms. AST and ALT activities were activated in liver of treated animals. The disruption of transaminases from the normal values denotes biochemical important and lesions of tissues and cellular function because they are involved in the detoxification process, metabolism and biosynthesis of energetic macromolecules for different essential functions (Tordior and Van Heem Stra-Lequin, 1980). Animals in their living environments, ingest, inhale, and absorb many chemicals that can impose stress on the organism and trigger tissue damage by numerous biochemical mechanisms. Since the liver is a primary site of biotransformation of foreign compounds, it is particularly vulnerable (Shalaby, 2006; Shalaby and Abdou, 2020). The changes occurred in kidney function parameters (urea and creatinine) with chemical fungicide were in form of highly significant increase of urea and creatinine; may be due to epithelial necrosis to the renal tubules with nuclear and chromatin changes in the epithelium of cortical tubules (Janssen, 1984; Shalaby, 2006).

It is concluded that the bio-control agents are considered safe as appearing related to the natural ecosystems. Generally, the present results and former literatures or previous studies showed clearly that all chemicals, unfortunately, are toxic or caused bad effects on human, animals and environment, particularity it's used in pest control field without exception. Accordingly, the extensive and unwise use of synthetic chemicals in the control programs against agricultural pests of creates in major deleterious side effects. So, this research aimed to found a safe alternative for control plant pathogens. Therefore we recommend using tested bio-agent as alternative chemical fungicides.

Acknowledgements

Authors would like to thank In-house project office, National Research Centre, Egypt. This study was funded by National Research Centre, Grant No.12050131under title: Development and Large Scale Fermentation Manufacturing of Microbial Biofungicides for Control of some Plant Diseases, from 2019- 2022; PI. Wafaa M. Haggag.

References

Adjrah, Y., Karou, S. D., Agbonon, A., Eklu-gadegbeku, K., de Souza, C. and Gbeassor, M. (2013). Toxicological assessment of effect of mancozeb lettuce (*Lactuca sativa*) on Wistar rat liver. Ethiopian Journal of Environmental Studies and Management, 6:67-73.

Anonymous (2012). A World Compendium, The Pesticide Manual Sixteenth Edition.

Betsabee, O., Luis, S., Arturo, R. and Montserrata, R. (2017). Evaluation of the toxicity and pathogenicity of biocontrol agents in murine models, chicken embryos and dermal irritation in rabbits. Toxicological Research, 6:188-198.

Cohort Software (1986). Costat user's manual virgin 3.03. Berkley. California, U.S.A.

Duke, K. A., Becker, M. G, Girard, I. J., Millar, J. L., Dilantha Fernando, W. G., Belmonte, M. F. and de Kievit, T. R. (2017). The biocontrol agent Pseudomonas chlororaphis PA23 primes Brassica napus defenses through distinct gene networks. BMC Genomics, 18:467.

Fei Law, W., Leng Ser, H., Khan, T., Hong Chuah, L., Pusparajah, P., Gan Chan, K., Hing Goh, K. and Han Lee, L. (2017). The Potential of *Streptomyces* as Biocontrol Agents against the Rice Blast Fungus, *Magnaporthe oryzae* (*Pyricularia oryzae*). Frontiers in Microbiology, 17.

Gromysz, M. (1993). Substrate specificity of mouse-liver microsomal enzymes in S-fenvalerate metabolism *ACS* Symposium series No. 42, synthetic S-envalerate. American Chemical Society, Washington, D.C.

Haggag, W. M. and Ali, R. R. (2019). Microorganisms for wheat improvement under biotic stress and dry climate. Agricultural Engineering International: CIGR Journal, 21:118-126.

Haggag, W. M. and Abouziena, H. F. (2016). Improved caroteno-protein and exopolysaccharide production by *Rhodotorula glutinis* for management of wheat grain diseases. Ponte Academic Journal, 72:79-107.

Haggag, W. M., Abouziena, H., Abd El Wahed, Hoballah, E. and Islam, A. (2015). Application of blue-green algae for integrated disease management of barley against foliar pathogens. Journal of Chemical and Pharmaceutical Research, 7:266-272.

Janssen, W. (1984). Forensic Histopathology. Spring- Verlag, Berlin, NY, pp.314-315.

Madbouly, A. K. (2018). Efficacy of Pseudomonads as biocontrol agents of phytopathogens. Novel Research in Microbiology Journal, 2:48-52.

Ohnishi, Y., Ishikawa, J. and Hara, H. (2008). Genome sequence of the streptomycin-producing microorganism *Streptomyces griseus* IFO 13350. Journal of Bacteriology, 190:4050-4060.

Schalm, O. W. (1986). Veterinary Hematology. 4th Ed., Loa and Fibiger, Philadelphia, pp.21-86.

Shalaby, Sh E. M. (2006). Comparative haemato and hepatorenal toxicity of IGR, Lufenuron and Profenofos insecticide on albino rats. JEgyptian Society of Clinical Toxicology Journal, 34: 85-98.

Shalaby, Sh E. M. and Abdou, G. Y. (2020.) Assessment of Pesticide Residues in Blood Samples of Workers at Agriculture Activities in Egypt. Journal of Plant Protection Research, 60:369-376.

Tordior, W. F. and Van Heem Stra-Lequin, E. A. (1980). Field studies monitoring exposure and effects in the development of pesticides. Elsevier, Amsterdam, Oxford, New York: 207.

Umechuruba, C. I. and Nwachukwa, E. O. (1997). The effect of filtrates of seed borne fungi of African yam bean on seed germination and seedling development. Global Journal of Pure and Applied Sciences, 3:165-176.

Xiao, Y., Li, H. X. and Li, C. (2013). Antifungal screening of endophytic fungi from *Ginkgo biloba* for discovery of potent anti-phytopathogenic fungicides. FEMS Microbiol Lett 2013; 339:130-6.

Yahia, E., Aiche, M. A., Chouabbia, A. and Boulakoud, M. S. (2014). Subchronic mancozeb treatment induced liver toxicity via oxidative stress in male wistar rats. Comm. Applied Biological Sciences, Ghent University, 79:553-559.

(Received: 10 September 2022, accepted: 20 October 2022)