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Combined Effect of a Chitinase Producing Bacteria and *Bacillus thuringiensis* Against *Musca domestica* (Diptera: Muscidae) Larvae.

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Abstract: In the current study we investigated the potential of *Streptomyces griseus* which is a chitinase producing bacteria for insect control as a bio-pesticide and its mixture with *Bacillus thuringiensis* as a different mode of action. This S*treptomyces griseus* was isolated from Elsulayel area, Saudi Arabia at 2011. Serial dilution technique was adopted to isolate the organism and was screened for its chitinolytic activity. The activity of chitinase extracted was determined using standard colloidal chitin as the reference control. The enzyme activities were found to be 0.12 µg/ml/minute for degradation of crab. *Musca domestica* L. (Diptera: Muscidae) larvae was tested in feeding, drinking and contacts toxicity methods with both *Streptomyces griseus* and *Bacillus thuringiensis*, either separately of in combination with each other. Reduction in the percentages of pupal and adult emergence was recorded for seven days after treatment by two doses of *B. thuringiensis* (5.0 and 1.0 mg/ L) and with suspensions containing approximately 5×10^{10} ml^{-1} of *Streptomyces griseus*. The chosen concentrations were 1, 0.5 & 0.1 µmg/ ml. Contact toxicity of the *Streptomyces griseus* was found very weak and no significant result in comparison with normal control except in younger instars only. The bacterium *Bacillus thuringiensis* proved to be a good candidate in controlling *Musca domestica*. Furthermore, treated extracts affected house fly larvae and resulted in reduction of adult stages. The number of tested larvae developed to pupae and adults was less than that of control group. The crude extract at concentration $9.5x10^4$ mg/l completely inhibited development from larvae to pupae. The current study gives the attentions that the *Streptomyces griseus,* which is a chitinase producing bacteria is not promising to be used as a bio-control agent or as an additives to *Bacillus thuringiensis* in reduction of *Musca domestica* population in control managements strategy. The difference of significance was found 4% to 5% in larval mortality with the first and second larval instars. The larvae was not susceptible to be affected and may be immunology resistant to *Streptomyces griseus,* non-pathogenic. It is only act as biodegradation factor for chitin in nature but very weak to act in alive biological system.

Key words: *Musca*, synanthropic fly species, bacterial control, *Bacillus thuringiensis*, *Streptomyces griseus.*

Introduction

House fly, *Musca domestica* L. (Diptera: Muscidae) is an ideal vector for transmission of many pathogens in human and animal because of its biology and ecology [1,2]. House flies can be found worldwide, except Antarctica. Many authors had reported that the fly species recovered in the highest numbers were *Musca domestica* L.[3] in Buraydah, Saudi Arabia,[4] in central New South Wales and central Queensland,[5] in Al Dhaid (UAE)[6]in central California,[7]in Zhejiang province, China,[8] in central New South Wales and central Queensland [9] at Wadi Eldawaser Saudi Arabia.Therefore, they become very important in public health concern. Chemical insecticides are commonly used for their control. Nevertheless, the widespread and inappropriate application of chemical insecticides produce the risk of developing pest resistance, side effects to non-target species, and long lived residues in the environment. That is why in need for reduction. Consequently, they had searched for saver methods of control[10].

The bacterium *Bacillus thuringiensis* proved to be a good candidate in controlling *Musca domestica*, associated with poultry houses, as stated by[11-19]. *B. thuringiensis* is safe to human and farm animals [20] in addition to non-target many beneficial species[21]. No resistance against this biological agent is recorded for disease transmitting vectors[22]. *B. thuringiensis* Berliner fed to chicken is reported to inhibit the development of *M. domestica* in chicken feces[17,18]. Concerning to mode of action *B. thuringiensis* is known to produce several toxins during its logarithmic phase of growth, the heat labile, α-exotoxins (Lecithinase C) and β-exotoxin which are water-in soluble and heat stable and highly toxic to the larvae of flies, and is applied to control flies in Russia [23].

This is the first study to clearly demonstrate the potential of the *Streptomyces griseus* which is a chitinase producing bacteria to be used against house flies as vectors for the transmission of pathogenic bacteria. As chitin occurs in insects as a major component of the cuticle and as protective lining the gut of many insects as well.

The present study (which is an extension of the work of [1] at Wadi Eldawaser Saudi Arabia) aimed to test the susceptibility of house flies *Musca domestica* larvae to *Streptomyces griseus* either alone or in combinability with *B. thuringiensis* or both of them independent and dependent manner as a different mode of action.

Experimental

1- Insects tested

House fly adults were collected from Benha city Qalubyia Governorate at 2011, and identified by the Department of Entomology, Faculty of Science, Benha University. Adult flies were kept and colonized in 0.3x0.3x0.3 m cages. They were reared with sugar, water soaked into cotton wool, and a combination of the synthetic rearing medium for immature stages. A laboratory prepared media of yeast extract and agar (after being softened with water) according to the method described by Shoukry and Radi[24] was used for rearing immature stages of *M. domestica*. This colony provide us with eggs and immature stages, at a weight ratio of 1:2 as a food source and an oviposition site. The food was changed daily. This laboratory colony of *M. domestica* was raised in controlled temperature (27 ± 2 °C) and humidity at 70 ± 2 RH. These provided us with eggs and larvae for tests and control experiments. To avoid the interaction between the tested pathogens and normal bacterial flora, bacterial pathogens were added in the same way to the flasks containing the tested larvae reared under aseptic conditions. Confirmatory tests were done to detect other contaminants whose colonies similar or foreign infection, samples from each specimen were incubated into Klinger`s iron agar (KIA) and confirmed by API 20E microtubes. When contamination was detected, the results were discarded and repeated.

2- Bio assays

Direct feeding infectivity experiments

Fifty gm of feeding media were mixed with different concentrations of *Streptomyces griseus* or *B. thuringiensis* either alone or in combinations (0.1, 0.5, 1.0 µl/g media) and distributed in experimental jars, seeded with sixty *Musca* eggs and incubated at 27 ± 2 °C and 70 ± 2 RH. Each experiment was repeated three times. Percentages of larval, pupal and adult reduction were calculated. Control experiment was carried out in

the same conditions but without *Streptomyces griseus* or *B. thuringiensis* contamination. Another reference control was done with each bacterial tested alone. The cages were washed daily and contamination was avoided.

Topical application of infectivity experiment

Two oral doses of *B. thuringiensis* concentration (1.0 and 5.0 mg/L dose) were used to feed larvae, from both experimental and control groups were collected daily and distributed in sterile jars (40 g/jar). Sixty fresh laid house fly eggs were seeded on the surface of each jar. Jars were incubated in the same conditions as insect rearing conditions. ANOVA test was used to analyze our data.

Toxicity of house fly larvae based on LC_{50} and LC_{90}

The bacterial different concentration was immediately prepared as previously described. Twenty house fly larvae were randomized for each replication of the 1st, 2nd, and 3rd instar larvae. Three replications were set up per treatment at room temperature (30ºC). First, test larvae were placed into one side of a Petri dish (0.1x0.015 m) and control without bacteria only in rearing medium. The container containing food of larvae was covered with muslin cloth and tied with rubber ring. Finally, the mortality of larvae was checked by softly touching each one with a small paint brush (No. 0) at 0, 24, 4 8 and 96 hours, respectively; and those which showed no response were considered dead. Mortality was calculated using Abbot's formula. The lethal concentration values (LC_{50} and LC_{90}) at different exposure time start from 48, and till the day seven of treatment. They were determined by EPA Probit Analysis Program. Later, all living larvae were studied further for their development to count the adult reduction. Another test was done to elucidate the effect of toxicity test of house fly by the appropriate concentration range for contact toxicity. Three replications were carried out per treatment (concentration).

Breeding media

The tested formulations were mixed with the rearing media for immature stages of *M. domestica*. Fifty gm of the rearing medium were distributed in glass jars for egg seeding as a control and also with the tested formulation. This medium were covered with sterile saw dust for pupation. The glass jars were used in testing and in control experiments and were covered with muslin to avoid foreign eggs to be deposited i.e. to avoid contamination. Sterile saw dust was used for pupation. The test and control experiments were incubated at 27 ± 2ºC and 70 ± 2 RH. Normal percentages of larval, pupal and adult reduction were calculated for both test and control, either treated with *Streptomyces griseus* either alone or in mixture with *B. thuringiensis* or both of them.

Bacterial stock

The *Streptomyces griseus* isolates samples were isolated from Elsulayel area, Saudi Arabia at 2011. The collected samples were mixed with Ca CO_3 and followed by sieving in 4 mm mesh screen. One gram of this isolates was stirred in 100 ml sterile water and serial dilution until 10 dilution had been made. One ml of each dilution was spread on Petri-dish containing starch nitrate agar medium. The dishes were incubated for 7 days at 28ºC till *Streptomyces griseus* colonies appeared. The purification was achieved by picking up of unique single identical morphological *Streptomyces griseus* colonies. These isolates were sub-cultured on specific medium and stored at 4ºC. *Streptomyces griseus* isolates were inoculated into starch liquid medium and incubated at 28 ± 2ºC for 2 weeks under stirred conditions. The developed growths were harvested by centrifugation at 5000 rpm and determined cellular proteins using bovine serum albumin (BSA) as a standard protein. Protein concentration was determined by using bovine serum albumin. A laboratory preparation of *Streptomyces griseus* by culturing on LB media, incubated over night at 27 ± 2 °C. and the growing cells were collected through centrifugation at 4000 rpm for 10 min. *Bacillus thuringiensis* serotype H-1 was used in form of a commercial formulation, supplied by the manufacture Bakthane L-69, Rohn and Haas Co. The *Streptomyces griseus* bacteria were solubilized in sterile distilled water to stock solution, also the broth extracted i.e. chitinase enzyme. The stock solutions were diluted to made different concentration. 0.5 µL was applied to the thorax of each *M. domestica* larva using a 700 series syringe and a PB 600 repeating dispenser (Hamilton, Reno, NV, USA). Ten of *M. domestica* larvae each were treated with one of the tested concentrations that were tested for each pervious preparation. After the topical application, larvae were transferred back into a plastic assay cup, held as described above, and provided with a larvae rearing medium.

Mortality data was recorded at 24, 48 and 72 h post topical application for determination of LD_{50}. Larvae treated with sterile distilled water was used as a positive control. Three replicates were completed.

The statistical analysis

The statistical analysis was performed using SPSS version 20. The data were analyzed using Analysis of Variance (ANOVA) and Duncan's Multiple Range procedures[25].

3- Enzyme Chitinase Production

For the production of chitinase enzyme was achieved by picking up of unique single identical morphological *Streptomyces griseus colonies*, which were sub-cultured on specific medium. These *Streptomyces griseus* isolates was grown in 100 ml of fresh medium (3% w/v chitin; 0.1% KH_2PO_4; 0.05% $MgSO_4.7H_2O$; 50mM Sodium Phosphate buffer, pH 6.0) in a 250 ml Erlenmeyer flask at 30°C. Control was done for the growth of the culture in this medium using blank as medium in which no inoculums was added. The supernatant (chitinase enzyme) was collected from 3 day old cultures by centrifuging the mixture at 12,000 g for 20 minutes. The enzyme was concentrated by condensing the solution in order to reduce its volume.

Measurement of chitinase enzyme activity

Chitinase activity was measured separately with colloidal chitin, chitin from crab shells (Fluka, Biochemika, UK.) was used as the substrate. The specific activity of chitinase (µg/ml/minute) was determined. Enzyme solution (0.5 ml) was added to 1.0 ml of substrate solution, which contained a 1.5 % suspension of the colloidal chitin prepared in a phosphate buffer (50mM, pH 6.0) separately and the mixture were incubated at 37°C for 15 minutes. After centrifugation, the amount of reducing sugars produced in the supernatant was determined by the Dinitro salicylic acid method for estimation of reducing sugars according to Miller method[26]. Using N-acetyl glucosamine as a reference compound according to Imoto and Yagishita[27]. One unit of chitinase activity was defined as the amount of the enzyme that produced 1 µ mol of reducing sugar per minute. The results of specific activity of chitinase are represented in Fig. 3.

Results

Results of the isolation and identification of the *Streptomyces griseus* bacterial species are presented in (Fig 1 and 2). Clear zones was observed indicated that the *Streptomyces griseus* bacteria had a chinolytic activity. Enzymatic assay proved the extracted chitinase enzyme with Specific activity (0.12µ g/ml/minute) of chitinase used for degradation of chitin extracted from crab and the standard chitin was 0.089 µ g/ml/minute Fig. 3.

Preliminary test indicated that the pathogenicity of the tested bacteria against larvae of *Musca domestica* using contact method technique was very low. The toxicity bioassays with *M. domestica* larva either using *Streptomyces griseus* bacteria or the broth of its chitinase enzyme and their mixtures with *Bacillus thuringiensis* resulted in poor to no increase in activity for most of both preparation. In both sets of assays, the most active biotic agents was the *Bacillus thuringiensis* Table (1, 2&3).

This *Streptomyces griseus* colonies was tested against house fly larvae, *Musca domestica* L. (Diptera: Muscidae) and their development were determined by a dipping method and the chosen concentrations were 1, 0.5 & 0.1µmg/g. In the present investigation we tested *Musca domestica* larvae was tested in feeding and drinking methods with *Bacillus thuringiensis* and the chosen concentrations were 1&.5 µmg/ L . Contact toxicity of the *Streptomyces griseus* which is a chitinase producing bacteria was tested to house fly larvae, *Musca domestica* L. (Diptera: Muscidae) and their development were determined by a dipping method and the chosen concentrations were 1&5 µmg/ L. In the present investigation we tested the combinability of both of them independent and dependant manner as a different mode of action. Younger larvae were susceptible than older. Reduction in the percentages of pupal and adult emergence was recorded for seven days after treatment by two doses of *B. thuringiensis* (1&.5 µ mg L) and with two doses of *Streptomyces griseus*. The median lethal concentration (LC_{50}) values were calculated for 2nd instar larvae at 48 and 72 hours were indicated LC_{50} and LC_{90} values were 65-78 and 190- 220 µg ml-1 respectively. mg/l, respectively for *B. thuringiensis* and The LC_{50} and LC_{90} values were 1337.384 and 2070.516 µg/ml, for *Streptomyces griseus.*

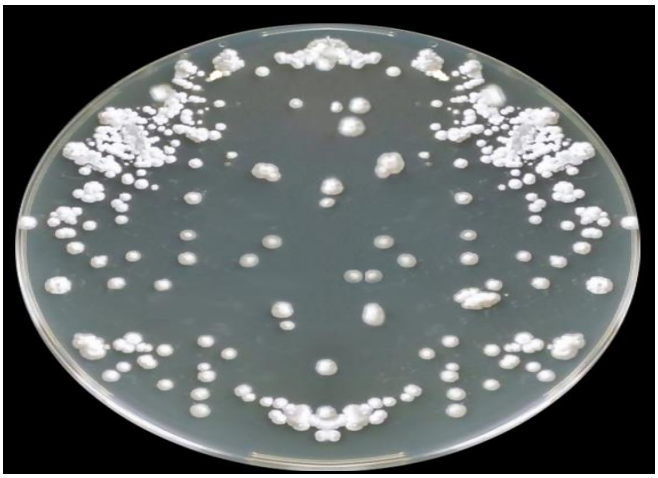

Fig.1: The *Streptomyces griseus* bacteria growing in media.

Fig. 2: Chitinase enzyme effect of *Streptomyces griseus* noted the clear zones (i.e. Chinolytic acitvity).

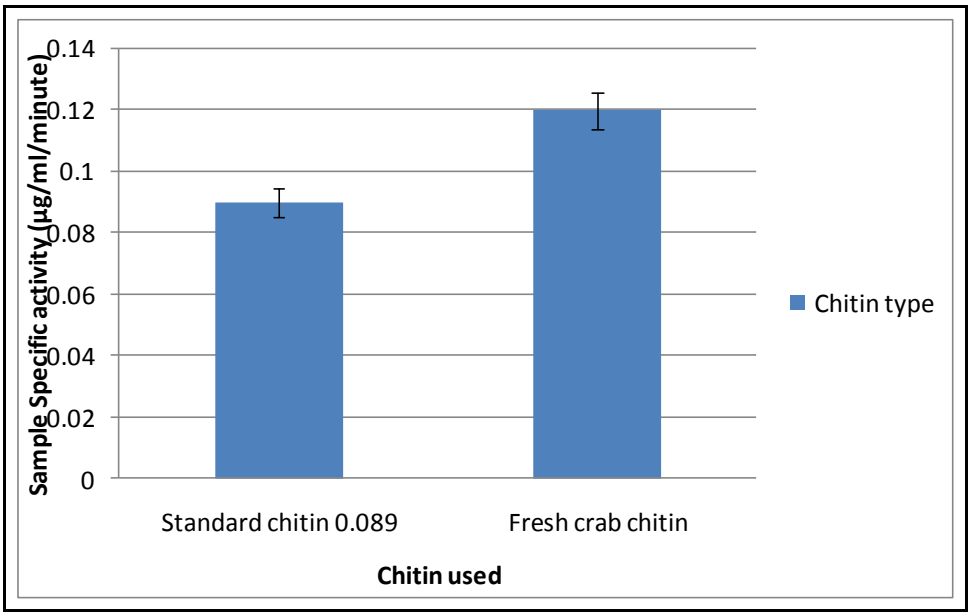

Fig.3: Specific activity (µg/ml/minute) of chitinase of *S. griseus* used for degradation of chitin extracted from fresh water crab and standard chitin.

Table (1) Effect of *Bacillus thuringiensis* (*B.t.*) and *Streptomyces griseus* (*S.g.*) on 2nd instar *Musca domestica* larvae after treatment for 48 h with LC_{50}. (66.5 & 99.5) respectively.

LC50 (µg ml-1)	No. of larvae	The LC_{50}		LC_{90}		Slope
		65.0 µg ml-l Mean ± S.D	95% CI	Mean ± S.D	95% CI	
B.t.	60	65.5 ± 1.1	(65-78)	200 ± 10.5	(190-220)	0. 576± 0.03
S.g. 5×10^{10} ml^{-1}	60	1337.384	(1337-1339)	2070.516	(2090-20100)	0.985±0.09
B.t.+S. g.	60, 2nd	60 ± 1.0	(63-70)	82 ± 1.6	(185-215)	
B.t.+S. g.	60, 3nd	61.0 ± 1.0	(62-71)	82 ± 1.6	(186-215)	
Control	60	98± 1.0	98	95± 1.0	95	

Table (2) Effect of *B. thuringiensis* (*B.t.*) on *M. domestica* larvae for prolonged time of exposure (7days).

Dose µg ml^{-1}	Mean larval mortality (%)					
	2d	3d	4d	5d	6d	7d
1	$0(0)^a$	$22.66(0.54)^a$	$35.67(0.65)^a$	$44.22(0.71)^a$	$44.34(0.73)^a$	$47.68(0.75)^a$
10	$11(0.32)^b$	$44.33(0.72)^b$	$51.00(0.79)^b$	$58.88(0.87)^b$	$62.12(0.90)^b$	$67.67(0.96)^b$
50	$21(0.46)^c$	$46.56(0.74)^b$	$62.00(0.89)^c$	$68.88(0.97)^c$	$72.12(1.01)^c$	$73.45(1.04)^b$
100	$32(0.58)^d$	$57.66(0.84)^c$	$67.66(0.96)^c$	$77.77(1.07)^d$	$83.42(1.14)^d$	$87.66(1.20)^c$
Control	0	2.0	3.0	5.0	5.0	5.0
F-Ratio	71.22	20.18	16.97	37.30	28.78	26.66
LSD	0.097	0.096	0.101	0.0714	0.102	0.115
CV %	14.1	6.8	6.6	4.8	5.9	6.2
Effect	**	**	**	**	**	**

-Mean followed by the same letter in the same column are not significant at P <0.05
- ** Highly significant with P<0.001.

Table (3) Effect of *Bacillus thuringiensis* (*B.t.*) and *Streptomyces griseus* (*S.g.*) on *Musca domestica* larvae after treatment for 48 h with LC_{25}. (35.0 & 50.0) respectively.

Fly breeding media	No. of larvae	No. of developed pupae		No. of emerged adults	
		Mean ± S.D	%	Mean ± S.D	%
B.t.	100	84 ± 1.1	84	77 ± 1.5	77
S.g.	100	94± 1.2	94	90±0.5	90
B.t.+S. g.	100	80 ± 1.0	80	70 ± 1.6	70
Control	100	98± 1.0	98	95± 1.0	95
Significance (S)		(S) P > 0.05		(S) P > 0.05	

Table (4) Effect of *B. thuringiensis* (*B.t.*) and *S. griseus* (*S.g.*) on total protein and lipids *contents* of 2nd instars *M. domestica* larvae after treatment for 48 h and 120 h. with LC_{50}. (66.5 &1337.5 µg/ml) respectively.

Bt commercial formulation *	Protein (mg/b.wt)* (mean ± SE)		Lipids (mg/b.wt) (mean ± SE)	
	48 h	120 h	48 h	120 h
Control	48.53 ± 0.61^a	35.31 ± 0.45^a	17.45 ± 0.41^b	15.41 ± 0.48^b
B.t.	51.76 ± 1.78^a	27.45 ± 0.25^b	16.22 ± 1.42^a	12.1 ± 0.75^b
S.g.	47.8 ± 0.44^a	36.56 ± 0.45^a	13.25 ± 0.44^b	13.35 ± 058^b
B.t.+S. g.	50.85 ± 0.41^a	27.48 ± 0.49^b	15.1 ± 0.91^a	13.22 ± 0.79^b

* b.wt = body weight.

Vertically means bearing different letters are significantly different at P≤0.001

Values within a column followed by different lowercase letters were significantly different (P≤0.05), while values within a column followed by the same lowercase letters were not significantly different (P≤0.05).

Furthermore, treated extracts affected house fly development in pupal and adult stages. The number of test larvae developed to pupae and adults was less than that of control group. The bacterium *Bacillus thuringiensis* proved to be a good candidate in controlling *Musca domestica*. Reduction in the percentages of pupal and adult emergence was recorded for six days after larvae feeding by two doses of *B. thuringiensis* (1.0 and 5.0 mg/ L) Table 5.

The concentration $8.5x10^4$ mg/l completely inhibited development from larvae to pupae. The current study gives the attentions that the *Streptomyces griseus*. which is a chitinase producing bacteria is not promising to be used as a bio-control agent or as an additives to *Bacillus thuringiensis* in reduction of *Musca domestica* population in control management's strategy. It is only act as biodegradation factor for chitin in nature but very weak to act in alive biological system.

Table (5) Mortality (%) of house fly (*M. domestica*) larvae treated with *B. thuringiensis* (*B.t.*) concentrations. (1.0 & 5.0mg/l) respectively and its combination with *S. griseus* (*S.g.*) with suspensions containing approximately 5×10^{10} $ml^{-1,}$ (prolonged time of exposure) from one day to six days post feeding.

Post feeding days	No. larvae Tested	*B. Thuringiensis* dose (mg/L)		No. developed pupae mean ± SD	Pupal reduction %	Adult reduction	
						Emerged from pupae mean ± SD	Total (R) %
1st	60	1.0	*B.t.*	50 ± 0.7	17≠	41 ± 0.6	32.8
	60		*B.t.*+*S. g.*	48 ± 1.1	20 ≠	40 ± 0.7	33
	60	5.0	*B.t.*	47 ± 1.8	21	38 ± 1.1	35
	60		*B.t.*+*S. g.*	46 ± 0.8	23**	38 ± 0.3	36
2nd	60	1.0	*B.t.*	39 ± 1.1	35**	30 ± 1.5	51**
	60		*B.t.*+*S. g.*	31 ± 1.6	48**	23 ± 1.5	61**
	60	5.0	*B.t.*	30 ± 1.9	49.8**	21 ± 0.3	65**
	60		*B.t.*+*S. g.*	28 ± 0.7	52.3**	19 ± 2.0	68**
3rd	60	1.0	*B.t.*	36 ± 1.4	41**	26 ± 0.6	56**
	60		*B.t.*+*S. g.*	34 ± 0.2	43.1**	23 ± 1.3	61.5
	60	5.0	*B.t.*	31 ± 0.7	47**	21 ± 0.3	65**
	60		*B.t.*+*S. g.*	28 ± 0.6	51.3**	20 ± 2.0	63.6**
4th	60	1.0	*B.t.*	53 ±1.5	11.7*	43 ± 2.1	28*
	60		*B.t.*+*S. g.*	51 ± 0.3	14.5*	42 ± 1.9	30*
	60	5.0	*B.t.*	50 ± 1.1	16.5*	40 ± 0.1	33.3*
	60		*B.t.*+*S. g.*	48 ± 1.3	19*	39 ± 1.4	35*
5th	60	1.0	*B.t.*	54 ± 0.8	12*	45 ± 0.5	24.5*
	60		*B.t.*+*S. g.*	53 ± 1.3	11.5*	44 ± 1.3	26*
	60	5.0	*B.t.*	54 ± 1.4	12.3*	42 ± 1.1	30*
	60		*B.t.*+*S. g.*	54 ± 1.1	14*	42 ± 0.6	30*
6th	60	1.0	*B.t.*	53 ± 1.3	11.7*	43 ± 1.4	29*
	60		*B.t.*+*S. g.*	54 ± 1.6	12*	43 ± .9	29*
	60	5.0	*B.t.*	54 ± 0.9	12	42 ± 1.4	30*
	60		*B.t.*+*S. g.*	55 ± 2.0	9≠	42 ± 0.8	30*
S. g.	60		*S. g.*	50 ± 2.0	16*	48 ± 2.0	20*
Cont.	60			55 ± 2.7	9	54 ± 2.0	10

** Highly significant (P<0.001).
*Significantly different (P<0.001).
≠Not significantly different (P≤0.05).

The susceptibility of house flies *Musca domestica* larvae to *Streptomyces griseus* either alone or in combinability with *B. thuringiensis* or both of them independent and dependant manner as a different mode of action was weak. The infectivity of the tested microrganisms (*Streptomyces griseus* either alone or in combinability with *B. thuringiensis*) to *M. domestica* was evaluated by two procedures: contact with the flies ("larvae +contact") and addition of the flies to the media ("larvae+ broth"). In both samples of larvae tested by either of each bateria or their mixtures. The susceptibility of *M. domestica* to the commercial (whole culture)

forms of *B. thuringiensis* H-1 was tested through recording mortality percentages, pupal and adult reduction. The median lethal concentration (LC_{50}) values was calculated. LC_{50} and LC_{90} values were 65-78 and 190- 220 µg ml-1 respectively. The results is the average of three bioassays with the standard error. Concentration of viable spores, as estimated by the number of colonies, was 7.5 x 10^9 spores g -1 for bti and with suspensions containing approximately 5× 10^{10} ml^{-1} of *Streptomyces griseus.* The LC_{50} and LC_{90} values were 1337.384 and 2070.516 µ g/ml, for *Streptomyces griseus.*

Data in Table 1 revealed the efficiency with a slight decrease in the number of developing pupae and adults in the case of using Bti and Sg. in rearing medium. The concentrations of each bacterial form (1,&5 µl/g media) were used to contaminate synthetic media used for rearing house flies. Data in Table 2 represent the effect of *B. thuringiensis* H-1 commercial and laboratory preparations of *Streptomyces griseus* bacteria on the development of immature stages as well as adult emergence, after seeding eggs on rearing medium.

Treatments proved that *Bacillus* pathogenicity against housefly immature stages as well as adult emergence. There is an increase in maggot mortality with increasing concentrations of *B. thuringiensis* during the treatments. Increase in maggot mortality is correlated with a decrease of the pupal development for both preparations, compared with the control experiment. The present study indicated that *Streptomyces griseus* addition to *B. thuringiensis* showed high significant decrease in lipid contents after 48 hrs of treatment than the control Table (4) but not significant at 120 h..

Younger larvae were susceptible and reduction in the percentages of pupal and adult emergence was recorded for seven days after treatment by different concentration of *B. thuringiensis* Table 2. Results in Table 5 revealed the effect of (1 &5 µmg/L) of *Bt*. and with a doses of 5× 10^{10} ml^{-1} of *Streptomyces griseus*. The mean of mortality for the number of developed pupae and percentage of adult reduction indicated that older larvae was found much resistant to the treatment.

Discussion

The present study indicated that the *Streptomyces griseus* is not considered as a promising control agent and as well could not considered as an additive to *B. thuringiensis* H-1 to increase its toxicity. *B. thuringiensis* H-1 proved to be a good bacterial control agent against house flies *Musca domestica* which is not only a nuisance pest, but also acts as an important mechanical vector for various pathogenic microorganisms, this was found to be in accordance with [17,18] and [28]. Using *B. thuringiensis* as food additive can significantly reduce house fly pupal and adult emergence in urban area to high extent with great safety. Although *Streptomyces griseus* laboratory preparations induced a4%-5% higher pathogenic effect in adults flies reduction only with younger instar larvae but older larvae were found highly resistant to its chinolitic effect and the difference was not significant. However, this laboratory preparations proved to be active in reaction with chitin. This a great chinolytic effect may be can act as biodegradable agent with direct effect with chitin in nature but cannot act in such way with alive biological system. Although *B. thuringiensis* at a concentration of 1 µl/g media could induce adult reduction and reached 94.3% and 100% for commercial preparations. Adding a higher concentration of *Streptomyces griseus*as a feed additive to *B. thuringiensis* give no increase in its efficacy, it may undergo physiological and immunological processes during its passage in larval alimentary canal that reduce or inhibit its infectivity. This may be attributed to the digestive enzymes of the insect gut inhibit the chinolitic activity of the *Streptomyces griseus* chitinase`s after ingestion which resulted in its inactivity. Lipids are the most suitable materials for storage of energy reserves. Compared to carbohydrates, lipids can supply as much as eight times more energy per unit weight [29]. In this study, *Streptomyces griseus* addition to *B. thuringiensis* showed high significant decrease in lipid contents after 48 hrs of treatment than the control Table (4). Similarly lipid content in tobacco cutworm *Spodoptera litura* (fab.) larvae was studied by Tripathi & Singh[30], the infection resulted in significant reduction in total lipid content of haemolymph in 3rd, 4th and 5th instars infected larvae. They suggested that the reason for the lower fat content in infected larvae could be the extended larval period of the treated insects and blocked food ingestion, and the fat reserves might have been utilized for the maintenance during extended larval period. Also Mehrabi, et al [31] reported that the *B. thuringiensis* protein crystal was toxic against larvae of the house fly. They also added that a concentration of 0.43 mg/mL of this toxin caused 100% mortality in house fly larvae. The LD_{50} amount of these toxins was calculated as 125 µg/g.

Sampson and Gooday[32] reported that the *Bacillus thuringiensis* subsp. *israelensis* IPS78 and *B.*

thuringiensis subsp. *aizawai* HD133 both secreted exochitinase activity when grown in a medium containing chitin. Allosamidin, a specific chitinase inhibitor, inhibited activity from both strains, with IC_{50} values of about 50 μ M with colloidal chitin as substrate and between 1 and 10 μM with 4-methyl umbelliferyl-diacetyl chitobioside and 4-methylumbelliferyl-triacetylchitotrioside as substrates. They investigated the involvement of these chitinolytic activities during pathogenesis in insects with *B. thuringiensis* subsp. *israelensis* IPS78 against larvae of the midge *Culicoides nubeculosus*, and with *B. thuringiensis* subsp. *aizawai* HD133 against caterpillars of the cotton leaf worm *Spodoptera littoralis*. Presence of 100 μ M allosamidin increased the LD_{50} by factors of 1.3 and 1.4, respectively, they demonstrated a role for bacterial chitinases in the attack on the insects. Presence of chitinase A from *Serratiama rcescens* considerably decreased the values for LD_{50}' confirming previous observations with different systems of the potentiation of entomopathogenesis of *B. thuringiensis* by exogenous chitinases. The most likely action of the endogenous chitinases of *B. thuringiensis* is to weaken the insects' peritrophic membranes, allowing more ready access of the bacterial toxins to the gut epithelia. Addition of exogenous chitinases will then increase this effect. Complementary cross-infection experiments, strain HD133 against midge larvae and strain IPS78 against caterpillars, were performed to investigate the pathogen/host specificities of the effects. Results showed that much higher concentrations of bacteria were required to achieve even low mortalities. Although our results indicated that the addition of a chitinase gave no increase in death rate.

In conclusion, *B. thuringiensis* H-1 is an environmentally safe biotic agent insecticide and proved its toxicity against different stages of house flies. *B. thuringiensis* H-1 proved its efficiency as feed additive to reduce pupal and adult percentages, this finding was similar with that found by Ali, [21] whom proved the safety of *B. thuringiensis*, as environmentally friendly agents to non-target invertebrates and vertebrates. The *Streptomyces griseus* have no chance to be used as bio-control agent.

In the future, there is much work to be done. Examples of some of the additional studies that are needed include research of the potential of the endoprastisic flora of house flies (Diptera: Muscidae) to be used as a target to suppress the digestion of the flies and which one is essential for doing such job.

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