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Bacillus thuringiensis as a feed additive to control *Musca domestica* associated with poultry houses

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Abstract The bacterium *Bacillus thuringiensis* proved to be a good candidate in controlling *Musca domestica*, associated with poultry houses, as a carrier of a wide range of pathogens infecting man and animals. Chicken feces are good media attracting flies for breeding. The bacterium was used in commercial form and a laboratory preparation form to contaminate feces or administered orally to chicken. Reduction in the percentages of pupal and adult emergence was recorded for six days after chicken feeding by two doses of *B. thuringiensis* (1.00 and 5.00 mg/ kg).

Introduction

High distribution of poultry houses in rural and urbanizing areas increase the breeding places of the housefly *Musca domestica*. Accumulation of chicken feces is considered very rich media for housefly rearing (WHO, 1997). With the movement of people and housing different habitats, fly dispersing from livestock becomes a significant public health problem, (Gingrich, 1995; Chavasse et al., 1996; De Jesus et al., 2004; Tally et al., 2009; Greig et al., 2010; Butler et al., 2010). House fly is incriminated to transmit the worldwide hemorrhagic bacteria *Escherichia coli* (0157:H7) which causes an outbreak in many countries in Asia and Europe (Iwasha et al., 1999; Alam and Ludek, 2004; Butler et al., 2010). The reduction of house fly populations using the microbial agent *Bacillus thuringiensis* had begun early in 1958, when feeding tests were done in the laboratory to determine the susceptibility of the house fly *M. domestica* to strains of *B. thuringiensis* which is considered a promising biological control agent against vectors of diseases (Briggs, 1960; Borgatti and Guyer, 1963; Saleh, 1989; Park et al., 2007; Sharma et al., 2008; Otieno-Ayayo et al., 2008) *B. thuringiensis* is safe to human and farm animals (Krieg et al., 1980) and to non-target many beneficial species (Ali, 1981). No resistance against this biological agent is recorded for disease transmitting vectors (Cariberg and Lindstrom, 1987). *B. thuringiensis* Berliner fed to chicken is reported to inhibit the development of *M. domestica* in chicken feces (Hodgman et al., 1993; Mwamburi et al., 2010). *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, which has the name (Thuringiensin) and is applied to control flies in Russia (Toumanoff, 1956).

The present investigation is aimed at evaluating the application of *B. thuringiensis* H-1 (Commercial and Laboratory Preparations) to control the house fly, *M. domestica* associated with poultry houses.

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Materials and methods

Colonization

Laboratory colony of *M. domestica* was raised in an insectary of controlled temperature (27 ± 2 °C) and humidity at 70 ± 2 RH, according to the method of Shoukry and Radi (1988).

Breeding media

(A) *The synthetic medium:* A laboratory prepared media of yeast extract and agar according to the method described by Shoukry and Radi (1988) was used for rearing immature stages of *M. domestica*.
(B) *Chicken fecal medium:* Fecal parts from chicken breeding cages were collected daily and used as rearing media for immature stages of *M. domestica*. Fifty gm of fecal parts were distributed in glass jars for egg seeding. Feces were covered with sterile saw dust for pupation. Jars were covered with muslin to avoid foreign eggs and incubated at 27 ± 2° and 70 ± 2 RH. Normal percentages of larval, pupal and adult reduction were calculated for both media.

Bacteria

Bacillus thuringiensis serotype H-1 was used in two forms, a commercial formulation, supplied by the manufacture Bakthane L-69, Rohn and Haas Co. and a laboratory preparation by culturing on LB media, incubated overnight at 27 ± 2 °C. Growing cells were collected through centrifugation at 4000 rpm for 10 min.

Bio assays

Direct infectivity experiments

Fifty gm of hen feces were mixed with different *B. thuringiensis* concentrations (0.1, 0.5, 1.0 µl/g media) and distributed in experimental jars, seeded with fifty *Musa* 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 *B. thuringiensis* contamination. Chicken cages were washed daily, assuming that fresh 24-h feces were collected daily.

Indirect infectivity experiment

Two oral doses of *B. thuringiensis* concentration (1.0 and 5.0 mg/kg weight) were used to feed hens, of one kilogram directly. Feces from both experimental and control groups were collected daily and distributed in sterile jars (40 g/jar). Fifty 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.

Results

The susceptibility of *M. domestica* to the commercial and laboratory (whole culture) forms of *B. thuringiensis* H-1 was tested through recording mortality percentages, pupal and adult reduction. Data in Table 1 revealed almost similar breeding efficiency with a slight decrease in the number of developing pupae and adults in the case of using chicken fecal medium. Three different concentrations of each bacterial form (0.1, 0.5, 1.0 µl/g media) were used to contaminate chicken fecal parts or synthetic media used for rearing house flies. Data in Table 2 represent the effect of two forms of *B. thuringiensis* H-1 (commercial and laboratory preparations) on the development of immature stages as well as adult emergence, after seeding eggs on fecal parts. Treatments proved *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 two treatments. Increase in maggot mortality is correlated with a decrease of the pupal development for both preparations, compared with the control experiment.

Maggots' mortality readings were recorded as 26.6%, 53.3%, and 84.4%. This reduction is highly significant when compared with the normal reduction in the control experiment (11.6%). Maggot reduction after rearing on *B. thuringiensis* (laboratory preparation) contaminated feces is slightly higher than the reduction after using commercial formulation, recording 34.2%, 57.5%, 87.5% after treating with *B. thuringiensis* concentrations at 0.1, 0.5 and 1.0 µl/g media. Percentage number of developed pupae is significantly decreased to be 73.3%, 46.6%, 13.3%; 65.8%, 42.5%, and 12.5% after rearing on media contaminated with the commercial *B. thuringiensis* formulation and laboratory preparations respectively, while percentages of developed pupae of the control experiment was 88.3%. The reduction is pupal formations ranged from 26.7% to 87.5%, Table 2.

The percentage of reduction of adult emergence was measured to be 64.2%, 70.8%, and 94.2% when contaminated feces was mixed with different concentrations of *B. thuringiensis* commercial formulation, and 65%, 75% and 100% after seeding housefly eggs on feces contaminated with *B. thuringiensis* laboratory preparations, while the reduction of adults was 20.6% in the control experiment.

Table 3 showed the development of houseflies reared on feces of hens previously fed on *B. thuringiensis* H-1. Two bacterial doses were used 1.0, 5.0 ml/kg. Percentages of pupal and adult reduction were recorded for six days post feeding. On the

Table 1 Efficiency of synthetic and chicken fecal parts as media for rearing *Musca domestica*.

Fly breeding media	No. of eggs	No. of developed pupae		No. emerged adults	
		Mean ± SD	%	Mean ± SD	%
Synthetic	120	102 ± 1.70	85	94.00 ± 1.7	78.30
Fecal parts	120	108 ± 1.00	90	98 ± 1.8	81.70
Significance (S)		(S) $P > 0.05$		(S) $P > 0.05$	

Table 2 Effect of commercial formulation and laboratory preparations of *B. thuringiensis* H-1 on different stages of housefly *Musca domestica* reared on chicken feces.

B. thuringiensis H-1 concentrations µl/g media		No.of eggs	Maggot mortality		Developed pupae			% Reduction of adult emergence	
			Mean ± S.D	%	Mean ± S.D	%	% Reduction (R)	From pupae	% (R)
0.1	Commercial	120	32 ± 1.7	26.6*	88 ± 2	73.3*	26.7	51.1	64.2**
	Lab. prep.	120	41 ± 1.0	34.2*	79 ± 1	65.8*	34.2	53.2	65**
0.5	Commercial	120	64 ± 1.0	53.3**	56 ± 1.7	46.6**	53.4	37.52	70.8**
	Lab. prep.	120	69 ± 1.0	57.5**	51 ± 2	42.5**	57.5	30.4.3	75**
1.0	Commercial	120	101 ± 1.5	84.4**	16 ± 3	13.3**	86.7	56.3	94.2**
	Lab. prep.	120	105 ± 1.0	87.5**	15 ± 1	12.5**	87.5	100	100**
$\overline{X}^2$			0.89		4.21			1.88	
Control		120	14 ± 0	11.6	106 ± 1	88.3		10.1	20.6

NS = non-significant.
* Significant ($P < 0.05$).
** Highly significant.

Table 3 Effect of *B. thuringiensis* H-1, as feed additives for chicken, against different stages of *Musca domestica* reared on feces.

Post feeding days	No. eggs	*B. thuringiensis* dose (mg/kg)		No. developed pupae mean ± SD	% Pupal reduction	Adult reduction	
						Emerged from pupae mean ± SD	Total (R) %
1st	50	1.0	Comm.	41 ± 0.5	18#	35 ± 1.6	30.5*
	50		Lab.	40 ± 1.8	18.8#	32 ± 1.5	36.0*
	50	5.0	Comm.	39 ± 1.7	19.2*	30 ± 1.1	40.2**
	50		Lab.	38 ± 0.5	19.5*	29 ± 0.5	42.0**
2nd	50	1.0	Comm.	29 ± 1.2	20**	20 ± 2.0	60.00**
	50		Lab.	22 ± 1.5	54.8**	18 ± 1.2	64.5**
	50	5.0	Comm.	20 ± 1.07	53.5**	17 ± 0.5	66.00**
	50		Lab.	19 ± 2.0	56**	15 ± 1.5	69.1**
3rd	50	1.0	Comm.	21 ± 0.2	58**	18 ± 2.0	64.5**
	50		Lab.	25 ± 1.1	50.5**	19 ± 1.8	62.5**
	50	5.0	Comm.	20 ± 0.5	60**	16 ± 0.5	68.0**
	50		Lab.	19 ± 1.2	58.8**	12 ± 1.2	76.5**
4th	50	1.0	Comm.	42 ± 1.07	18.5*	25 ± 0.7	50.8**
	50		Lab.	42 ± 0.05	18.8*	25 ± 0.7	50.8**
	50	5.0	Comm.	40 ± 1.5	19.5*	23 ± 1.5	54.2**
	50		Lab.	39 ± 0.6	20**	22 ± 1.5	56.6**
5th	50	1.0	Comm.	44 ± 0.1	15*	34 ± 1.7	32.2*
	50		Lab.	43 ± 0.2	14.7*	33 ± 2.6	34.0*
	50	5.0	Comm.	44 ± 2	15.5*	32 ± 1.22	36.5*
	50		Lab.	44 ± 2.5	16*	30 ± 0.00	40.1*
6th	50	1.0	Comm.	45 ± 1	15*	43 ± 1.5	14.8#
	50		Lab.	45 ± 0.5	15.8*	42 ± 0.8	16.1#
	50	5.0	Comm.	45 ± 0.1	10#	42 ± 0.5	16.5#
	50		Lab.	45 ± 0.05	10#	42 ± 1.5	16.3#
Cont.	50			46 ± 2.5	9	43 ± 1.5	14.2

$P < 0.001$.
* Significant.
** Highly significant.
# Non-significant.

first day, the percentage reduction in pupal formation was recorded to be 18% and 18.8% after using *B. thuringiensis* commercial and laboratory preparations as feed additives at the dose of 1.0 ml/kg. Reduction was higher with increasing *B. thuringiensis* dose to be 5.0 ml/kg, the recorded pupal reduction was 19.2% and 19.5%. The total adult reduction significantly increased to reach 30.5% and 36% for commercial and laboratory preparations at 1.0 ml/kg for the first day, corresponding to 14.2% normal reduction in the control experiment. The recorded adult reduction at 5.0 ml/kg was 40.2% and 42% for commercial and laboratory preparations. There was an increase in both pupal and adult reduction on the second day post treatment. Percentage of pupal reduction was 54.8% and 56% at *B. thuringiensis* doses of 1 and 5 ml/kg when hens were fed with laboratory *B. thuringiensis* preparations, while the reduction was 20%, 53.5% at the same doses after using *B. thuringiensis* commercial formulation as feed additive. A highly significant increase in percentage of adult

reduction was recorded on the second day and reached 64.5% and 69.1% when using laboratory preparations of *B. thuringiensis* at doses 1 and 5 ml/kg; while adult reduction reached 60% and 66% when using commercial formulation as a diet at the same *B. thuringiensis* doses. The percentage of pupal and adult reduction reached its maximum level at the third day where pupal reduction reached 58% and 50.5% for both *B. thuringiensis* preparations at 1.0 ml/kg and 60% and 58.8% for the concentration of 5.0 ml/kg. Adult emergence also reached its maximum reduction on the third day, recording 46.5% and 62.5% for both preparations at 1.0 ml/kg, and 68.0% and 76.5% at 5.0 ml/kg as doses.

A sharp decrease in pupal reduction was realized on the fourth day, recording 18.5% and 18.8% after treatment with the lower doses of commercial and laboratory preparations of *B. thuringiensis* while higher concentration reached pupal reduction reach 19.5% and 20%. Percentages of adult reduction are also decreased to be 50.8% and 50.8% for commercial and laboratory preparations when hens were fed on 1.0 ml/kg *B. thuringiensis,* while at a concentration of 5.0 ml/kg, the adult reduction was 54.2% and 56.6% for commercial and laboratory preparations, respectively.

The decrease in percentage reduction continued during the fifth day and reached its maximum during the 6th day where reduction in pupae reached 10% and 10.5% for the low and high doses for commercial and laboratory *B. thuringiensis* preparations. No significant difference was recorded between the reduction in *B. thuringiensis* treated media and control experiments (9%).

Reduction in adult emergence reached the lowest levels of 14.8% and 16.1% for a dose of 0.1 ml/kg and 16.5% and 16.5% at 5 ml/kg, for commercial and lab. *B. thuringiensis* preparations. The reduction was insignificant compared with the normal adult reduction i.e. 14.2%.

Discussion

B. thuringiensis H-1 proved to be a good microbial control agent against houseflies which are considered as vectors of many human and animal disease agents (Cariberg and Lindstrom, 1987; Hodgman et al., 1993; Gingrich, 1995; Jorg et al., 1999; Mwamburi et al., 2010). Using *B. thuringiensis* as feed additive can significantly reduce house fly pupal and adult emergence in poultry houses. Although *B. thuringiensis* laboratory preparations induced a higher pathogenic effect the commercial formulation the difference was not significant. Laboratory preparations may contain other metabolites that increase their toxicity. Although *B. thuringiensis* at a concentration of 1 µl/g media could induce adult reduction and reached 94.3% and 100% for commercial and laboratory preparations, we chose the higher concentration for use as hen feed additive, *B. thuringiensis* may undergo physiological and immunological processes during its passage in hen alimentary canal that reduce or inhibit its infectivity. *B. thuringiensis* was active in feces for four days, after ingestion, then suddenly its potentiality started to decrease. This may be attributed to the acidity of poultry feces. No disease symptoms or any disorders were realized for hens fed on *B. thuringiensis,* (Ali, 1981) proved the safety of such environmentally friendly agents to non-target invertebrates and vertebrates.

Conclusion

Houseflies preferred to breed on hen feces. Treatment of feces with biological control agents may help to control such vectors of diseases. *B. thuringiensis* H-1 is an environmentally safe bio-insecticide and proved its toxicity against different stages of houseflies. *B. thuringiensis* H-1 proved its efficiency as feed additive to reduce pupal and adult percentages of houseflies in poultry houses. Its use with repeated doses every four days is recommended.

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