

Malathion Resistance in *Tribolium castaneum* (Coleoptera: Tenebrionidae) in Bangladesh

Ataur Rahman^a, Md Shahjahan^a and Farid Talukder^{*b}

^aDepartment of Entomology, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh

^bDepartment of Crop Sciences, College of Agricultural and Marine Sciences, Sultan Qaboos University,
P. O. Box 34, Al-Khod 123, Oman

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Abstract. Malathion resistance in the red flour beetle (*Tribolium castaneum* Herbst) is widespread and stable in natural populations even in the absence of pesticide exposure. Nine sample populations of *T. castaneum* collected from nine different silos, central storage depots (CSD) and local storage depots (LSD) of Bangladesh, and were tested to determine their resistance ratio to Malathion. Insecticide resistance was observed in most of the collected populations. Among nine tested populations of *T. castaneum*, the Ghatail LSD population developed the highest level of Malathion resistance ratio, where as the Serajganj LSD population showed lowest resistance ratio at LC₅₀ level, 72 h after the treatment.

Keywords: insecticide resistance, *Tribolium castaneum*, Malathion, red flour beetle

Introduction

Synthetic insecticides are being used to control different stored-product insects in different parts of the world. As a result, the treated insect species are becoming more and more resistant to particular insecticides. Malathion was registered in 1958 and was considered the primary insecticide to control insect pests including red flour beetles in storage environments. After a few years, Malathion experienced widespread and severe resistance by stored grain pests. Malathion resistance in the red flour beetle, *Tribolium castaneum*, was first reported in early 1960s, for example from Nigeria in 1961 (Arnaud *et al.*, 2002; Hayward and Onyeard, 1962; Parkin *et al.*, 1962) and later, from different parts of the world, including the North America (Arnaud *et al.*, 2002; Haubruge *et al.*, 1997; Werner, 1997; Zettler and Arthur, 1997; Collins *et al.*, 1993; Zettler, 1991; Binns, 1986; Champ and Dyte, 1976). Malathion resistant strains of the red flour beetle, *Tribolium castaneum* were also reported from different Asian countries (Gibe and Motoyama, 2002; Dhaliwal and Chawla, 1997; Irshad and Gillani, 1990). Reports showed that adult red flour beetle, *Tribolium castaneum*, in Pakistan has developed 56-fold resistance and in the Philippines, a 57-fold resistance to Malathion (Gibe and Motoyama, 2002; Saleem and Shakoori, 1989). Different researchers reported that the Malathion-specific resistance in the *Tribolium castaneum* is widespread and stable in natural populations even in the absence of pesticide exposure (Arnaud and Haubruge, 2002).

The Bangladeshi farmers, grain dealers and consumers store their grains in granaries, receptacles and other containers. Grain

losses in storage due to insect attacks are often great. It was estimated that grain losses due to insect damage, during storage periods range from 5 to 10% of the world's total grain production. In Bangladesh, the annual grain damage caused by stored product insect-pests might be similar to the Indian annual losses of 6.5% of stored grains (Raju, 1984). Among the major insects, red flour beetle, *T. castaneum* (Herbst) is one of the most serious insect-pests, of various food and stored commodities and causes economic damage to both raw goods as well as finished products (Islam and Talukder, 2005; Husain, 1997; Zettler and Arthur, 1997; Irshad, 1995; Talukder and Howse, 1995). In Bangladesh, regular use of large quantities of synthetic insecticides including Malathion for stored-product protection is the key element of integrated pest management, which might cause residual toxicity in treated grains and also development of resistance in insects. A few research studies have been conducted to measure the level of insecticide resistance phenomenon under Bangladeshi storage conditions. The present study was undertaken to find out the Malathion resistance level in red flour beetle, *T. castaneum*, under different storage systems, through collection and bioassay of sample populations from 9 different areas of Bangladesh.

Materials and Methods

Wheat samples containing adult red flour beetles were collected from 9 different public storage locations in 7 districts of Bangladesh. The collected insects were reared and experiments on insecticide resistance were conducted in the Laboratory of the Department of Entomology, Bangladesh Agricultural University, Mymensingh, Bangladesh.

* Author for correspondence; E-mail: talukder@squ.edu.om

Sampling locations. Insects were collected from eight different public storage depots [i.e. Central Storage Depot (CSD) and Local Storage Depot (LSD)] and one silo each from different locations, namely - Narayanganj CSD, Kishoreganj LSD, Tejgaon CSD, Sheddiganj Silo, Tangail LSD, Ghatail LSD, Jamalpur LSD, Mymensingh CSD and Serajganj LSD located in different districts of Bangladesh. From collected information, it was found that all these CSD, LSD and silos had been using Malathion insecticide regularly, as part of their routine pest control strategy, at least for few years. The susceptible red flour beetle population was collected from a local source and reared for few generations under laboratory conditions under no influence of insecticide.

Insect sampling methods. The adult insects were collected from each food store, after detection of the infestation. Insect samples were kept separately in plastic bags which were labeled on the spot. Around 500-600 insects were collected from each storage facility. Collection was made by (a) sieving the commodity, (b) brushing the insects from floor, walls or surface of bags and (c) collecting small quantities of the commodity or residue with a sample collector (known as Bonga). After collection, insects were put into polythene bag with their food (wheat + broken wheat), providing proper aeration. They were carried to the Laboratory of Department of Entomology, Bangladesh Agricultural University, Mymensingh, for further processing.

Rearing of the test insect. The collected insects were sieved out from the sampling media after bringing them to the Laboratory. Wheat flour was used as insect rearing medium. 40-50 g of wheat flour were placed in plastic jars (9.5 cm x 7.5 cm) and 100-200 of the sieved out insects were then released in each jar for feeding and oviposition. The jars were placed in an insect rearing cage (55 cm x 38 cm). After a week, adults were removed from the wheat flour and the sieved food media were put back in the jars, and then incubated for 28-35 days for emergence of F₁ generation from them. The F₁ generation was used for the present experiments. The rearing temperature and the relative humidity of the insect cages were maintained at 27-30 °C and 70-75%, respectively, throughout the whole rearing and experimental period. In the case of susceptible population, 25 pairs of *T. castaneum* adults were received from the laboratory-reared susceptible culture, which was bred in the Entomology Lab. for many generations without any insecticide influence. For the present experiment, this susceptible population was also reared for one generation under the above controlled conditions to reproduce large number of F₁ progeny needed for the experiments.

Test insecticide. A commercial formulation of Malathion (trade name: Zithiol 57 EC, chemical group: organophospho-

rus, wide-spectrum, non-systemic insecticide and acaricide) was used for the present experiment.

Topical bioassay. Insects were tested for their resistance against Malathion, by topical application method of Talukder and Howse (1995) with few modifications. Different concentrations (2.00%, 1.00% and 0.5%) of the insecticide were prepared by diluting it with solvent (acetone). Adult beetles were chilled at 4 °C for a period of 10 min and then the immobilized insects were picked up individually. One µl (micro-litre) of diluted Malathion solution was applied to the dorsal surface of the thorax of insect using a micro-capillary tube. Fifty unsexed adult beetles, in 5 replicates of 10 insects each, were treated with each dose. In addition, the same numbers were treated with acetone (solvent) only as control. After treatment, insects were transferred to petri dishes (10 insects/petri dish) containing insect food (wheat flour). Treated insects were examined daily and those unable to move or respond to gentle touch were considered dead. Insect mortalities, were recorded at 24, 48 and 72 h after treatment (HAT).

Statistical analysis. The mortality data were corrected by Abbott's (1925) formula, transformed using arcsin percentage values and then were analyzed using factorial ANOVA. The mean values were adjudged by Duncan's multiple range test, DMRT (Duncan, 1951). Median lethal concentration (LC₅₀) and optimum lethal concentration (LC₉₀) were calculated using probit analysis (Finney, 1971) with a Log₁₀ transformation of concentration of insecticides. The resistance ratio (RR) were calculated on the basis of the following formula:

$$RR = (LC_{50} \text{ or } LC_{90} \text{ of resistance population} / LC_{50} \text{ or } LC_{90} \text{ of susceptible population})$$

Results and Discussion

Mortalities resulting from insecticide treatment in different red flour beetle population are shown in Table 1. The results showed that beetles treated with Malathion had lower mortalities, which in other words, proved that Malathion treatment has resistance problem. The Sheddiganj Silo population of red flour beetle, at 24, 48 and 72 HAT indicated that it possessed the highest resistance (2.00, 4.00 and 12.00% mortality, respectively) against Malathion, followed by the Tangail LSD population (4.00, 8.00 and 12.00% mortality, respectively). The Narayanganj CSD and Ghatail LSD beetle populations showed moderate-level of resistant (18.00% and 14.00% mortality at 72 HAT) to Malathion. On the other hand, population mortality rate of Serajganj LSD indicated that it possessed the highest susceptibility (43.11% at 72 HAT) to Malathion, followed by the Jamalpur LSD population (28.00% mortality at 72 HAT). Malathion showed high toxicity (mortality 68.89% at 72 HAT) to the susceptible population.

Table 1. Insect mortality rates due to Malathion topical application, collected from different places in Bangladesh

Sampling locations	Malathion dose (% a-i)	Insect mortality percent in different insecticides at*		
		24 HAT	48 HAT	72 HAT
Narayanganj CSD	2.00	6.00 fghi	16.00 defg	16.00 defg
	1.00	4.00 ghi	10.00 defghi	10.00 defghi
	0.50	0.00 I	2.00 I	4.00 hi
Kishorganj LSD	2.00	12.22 cdefgh	24.44 bcde	24.44 bcd
	1.00	8.00 efghi	14.67 defgh	14.67 defg
	0.50	0.00 hi	4.22 hi	6.22 fghi
Tejgaon CSD	2.00	6.00 fghi	16.00 defgh	18.00 defg
	1.00	6.00 fghi	12.00 defghi	18.00 defg
	0.50	2.00 hi	2.00 I	2.00 I
Sheddirganj Silo	2.00	4.00 ghi	8.00 fghi	12.00 defghi
	1.00	2.00 hi	2.00 I	4.00 hi
	0.50	0.00 I	2.00 I	2.00 I
Tangail LSD	2.00	6.00 fghi	8.00 fghi	12.00 defghi
	1.00	6.00 fghi	8.00 fghi	8.00 efghi
	0.50	2.00 hi	4.00 hi	4.00 ghi
Ghatail LSD	2.00	8.00 efghi	12.00 defghi	14.00 defghi
	1.00	4.00 ghi	8.00 fghi	10.00 defghi
	0.50	2.00 hi	4.00 hi	4.00 ghi
Mymensingh CSD	2.00	18.00 bcdef	20.00 defg	20.00 def
	1.00	10.00 defghi	16.00 defgh	18.00 defg
	0.50	2.00 hi	2.00 I	2.00 i
Jamalpur LSD	2.00	20.00 abcd	22.00 bcdef	28.00 bcd
	1.00	6.00 ghi	8.00 ghi	16.00 defgh
	0.50	4.00 ghi	8.00 efghi	10.00 defghi
Serajganj LSD	2.00	26.00 abc	38.89 abc	43.11 bc
	1.00	16.00 abcdef	20.22 cdefg	22.22 cde
	0.50	14.00 bcdefg	14.44 defgh	18.67 def
Susceptible	2.00	36.00 a	52.89 a	68.89 a
	1.00	30.00 ab	38.44 ab	45.55 b
	0.50	22.00 abcd	26.44 bcd	28.67 bcd

* = values followed by different alphabets (a-i) within a column are significantly different at 0.05 level (Duncan, 1951); * = original data were transformed into arcsin $\sqrt{\text{percentage}}$ values for the ANOVA test; HAT = hours after treatment; CSD = central storage depot; LSD = local storage depot

The results showed that among the nine different insect populations, the Sheddirganj Silo population possessed the highest resistance to Malathion, followed by Tangail LSD population. On the other hand, Serajganj population showed less resistant capability to Malathion. Werner (1997) reported that field strains of *T. castaneum* collected from various storages, bakeries and flour mills in the Czech Republic, showed different levels of resistance to organophosphate insecticides. Our results showed similar trends, where we found that red

flour beetle populations collected from nine different locations of Bangladesh had developed different levels of resistance to Malathion.

Probit analysis. The results on probit analysis for malathion show probit-mortality for nine resistant and one susceptible red flour beetle populations at 24, 48 and 72 HAT (Table 2-4). When compared with the susceptible population, most of the treated populations had higher LC_{50} as well as LC_{90} values. In case of LC_{50} values at 24 HAT, the

Table 2. Malathion toxicity of red flour beetle, 24 h after topical treatment (24 HAT)

Sampling locations	LC ₅₀ value (%)	95% fiducial limit	LC ₉₀ value (%)	95% fiducial limit	χ ² value	Slope ± SE
Narayanganj CSD	16.036	0.829-310.340	96.873	5.003-1873.922	0.938	1.639 ± 0.141
Kishoreganj LSD	15.439	0.665-359.243	157.776	6.786-3667.655	0.261	1.268 ± 0.114
Tejgaon CSD	48.819	0.204-1.169104	479.002	4.092-2.345X10 ⁵	0.225	0.983 ± 0.109
Sheddirganj Silo	19.226	0.445-831.823	105.185	2.432-4550.202	0.362	1.734 ± 0.181
Tangail LSD	86.915	0.002-4.356X10 ⁶	1576.68	0.031-7.907X10 ⁷	0.555	1.017 ± 0.191
Ghatail LSD	39.429	0.285-5468.615	591.119	4.267-8.201X10 ⁴	0.003	1.089 ± 0.110
Mymensingh CSD	6.448	1.819-22.853	35.677	10.067-126.444	0.376	1.723 ± 0.092
Jamalpur LSD	6.948	1.760-27.445	41.703	10.557-164.613	0.575	1.645 ± 0.107
Serajganj LSD	16.298	0.456-582.744	843.864	23.579-3.016X10 ⁴	0.261	0.747 ± 0.069
Susceptible	6.413	0.567-72.556	479.12	42.299-5415.903	0.032	0.683 ± 0.063

χ² = (Chi-square) test of goodness of fit (tabulated value is 3.84 with d.f. = 1, p<0.05); HAT = hours after treatment; CSD = central storage depot; LSD = local storage depot

Table 3. Malathion toxicity of red flour beetle, 48 h after topical treatment (48 HAT)

Sampling locations	LC ₅₀ value (%)	95% fiducial limit	LC ₉₀ value (%)	95% fiducial limit	χ ² value	Slope ± SE
Narayanganj CSD	8.007	1.662-38.585	52.420	10.879-252.588	0.536	1.569 ± 0.092
Kishoreganj LSD	5.965	1.390-25.648	43.142	10.045-185.376	0.280	1.490 ± 0.097
Tejgaon CSD	12.214	0.917-162.704	127.038	9.541-1691.990	1.151	1.259 ± 0.101
Sheddirganj Silo	19.226	0.455-831.823	105.185	2.432-4550.200	0.362	1.734 ± 0.181
Tangail LSD	54.511	0.051-832x10 ⁴	1224.439	1.145-1.31x10 ⁶	0.153	0.947 ± 0.126
Ghatail LSD	34.979	0.350-3497.65	820.987	8.208-8.199x10 ⁴	0.032	0.934 ± 0.090
Mymensingh CSD	5.702	1.791-18.154	20.002	6.283-63.688	2.211	1.632 ± 0.084
Jamalpur LSD	6.336	1.711-23.482	43.868	11.838-162.456	0.778	1.523 ± 0.083
Serajganj LSD	3.488	1.392-8.756	31.344	12.027-35.046	0.339	1.342 ± 0.070
Susceptible	1.617	0.947-2.760	20.528	1.145-1.31x10 ⁶	0.177	1.159 ± 0.063

χ² = (Chi-square) test of goodness of fit (tabulated value is 3.84 with d.f. = 1, p<0.05); HAT = hours after treatment; CSD = central storage depot; LSD = local storage depot

Table 4. Malathion toxicity of red flour beetle, 72 h after topical treatment (72 HAT)

Sampling locations	LC ₅₀ value (%)	95% fiducial limit	LC ₉₀ value (%)	95% fiducial limit	χ ² value	Slope ± SE
Narayanganj CSD	6.448	1.819-22.853	35.617	10.058-126.328	0.375	1.723 ± 0.092
Kishoreganj LSD	5.983	1.345-26.626	49.449	11.114-220.046	0.024	1.396 ± 0.091
Tejgaon CSD	9.550	1.142-79.844	95.881	11.475-802.125	2.436	1.278 ± 0.093
Sheddirganj Silo	6.013	0.925-39.090	18.269	2.812-118.840	0.011	2.652 ± 0.286
Tangail LSD	15.439	0.664-358.913	157.776	6.786-3667.655	0.261	1.268 ± 0.114
Ghatail LSD	19.675	0.746-519.406	321.825	12.193-8494.284	0.188	1.055 ± 0.087
Mymensingh CSD	4.886	1.853-12.882	25.993	9.863-68.550	1.879	1.763 ± 0.084
Jamalpur LSD	5.430	1.520-19.411	51.516	14.419-184.099	0.433	1.3909 ± 0.073
Serajganj LSD	3.114	1.274-7.611	33.337	13.644-81.482	0.748	1.243 ± 0.068
Susceptible	1.078	0.807-1.442	5.939	4.445-7.945	0.105	1.728 ± 0.065

χ² = (Chi-square) test of goodness of fit (tabulated value is 3.84 with d.f. = 1, p<0.05); HAT = hours after treatment; CSD = central storage depot; LSD = local storage depot

Tangail LSD beetle population was most tolerant to Malathion (LC_{50} 86.92%) treatment, followed by the Tejgaon CSD population (LC_{50} 48.82%). On the other hand, Mymensingh CSD population was the most susceptible (LC_{50} 6.45%) to Malathion treatment, among the treated populations, which indicates its highest susceptibility to Malathion. In case of LC_{90} values, the highest Malathion resistance was observed in the Tangail LSD beetle population (LC_{90} 1576.68%), followed by the Serajganj LSD population (LC_{90} 843.86%); Mymensingh CSD population showed the highest susceptibility (LC_{90} 35.67%) to the Malathion treatment (Table 2). At 48 HAT, in case of LC_{50} values, the Tangail LSD beetle population was the most tolerant (LC_{50} 54.51%) and Serajganj LSD population was the most susceptible (LC_{50} 3.49%) to Malathion. In case of LC_{90} values, though the highest Malathion resistance was observed in the Tangail LSD beetle population (LC_{90} 1224.43%), but the Mymensingh CSD population showed highest susceptibility (LC_{90} 20.00%) to the Malathion treatment (Table 3). But at 72 HAT, in case of LC_{50} values, the trend was changed and Ghatail LSD population was the most tolerant (LC_{50} 19.67%), followed by Tangail LSD. On the other hand, the Serajganj LSD beetle population was the most susceptible (LC_{50} 3.11%) to Malathion. When the LC_{90} values were compared, the highest Malathion resistance was also observed in the Ghatail LSD beetle population (LC_{90} 321.82%), followed by the Tangail LSD population, but the Sheddiganj silo population showed the highest susceptibility (LC_{90} 18.26%) to the Malathion treatment (Table 4).

Resistance ratio. The resistance ratios for 9 red flour beetle populations, collected from 9 different public storage facilities, are presented in Table 5. When compared, the *T. castaneum* population collected from the Ghatail local storage depot (LSD) showed highest malathion resistance ratio at both LC_{50} and LC_{90} levels (18.25 and 54.19, respectively), followed by the Tangail LSD population (14.32 and 26.57, respectively). On the other hand, the Serajganj LSD population showed the lowest resistance ratio at LC_{50} level (2.89) followed by Mymensingh CSD population (4.53), 72 h after treatment (HAT). It is interesting to notice that in case of Sheddiganj silo and Mymensingh CSD populations, at 72 HAT, the resistance ratios at LC_{90} levels were lower than their correlated values at LC_{50} levels. It might be associated with the higher LC_{90} values for the control (susceptible) population, as shown in Table 4.

Malathion resistance in the red flour beetle *T. castaneum* is a worldwide problem, and studies on resistance status in different countries are needed to improve insecticide resistance management (Haubruge *et al.*, 1997). The most important result of the present survey was the confirmation of different levels of resistance in *T. castaneum* to Malathion, in different parts of Bangladesh. Resistance to Malathion in this species presents a major problem to the grain storage pest control with this insecticide. Therefore, a better understanding of the response of *T. castaneum* species to the regular use of insecticide is required for the effective control of the pest.

Table 5. Resistance factor of Malathion at different hours after treatment (HAT) of red flour beetle

Sampling locations	Resistance ratio (RR)					
	LC_{50}			LC_{90}		
	24 HAT	48 HAT	72 HAT	24 HAT	48 HAT	72 HAT
Narayanganj CSD	2.50	4.95	5.98	<1.00	2.55	6.00
Kishoreganj LSD	2.41	3.69	5.55	<1.00	2.10	8.33
Tejgaon CSD	7.61	7.55	8.86	1.00	6.19	16.14
Sheddiganj Silo	3.00	11.89	5.58	<1.00	5.12	3.08
Tangail LSD	13.55	33.71	14.32	3.29	59.65	26.57
Ghatail LSD	6.15	21.63	18.25	1.23	39.99	54.19
Mymensingh CSD	1.01	3.53	4.53	<1.00	<1.00	4.38
Jamalpur LSD	1.08	3.92	5.04	<1.00	2.14	8.67
Serajganj LSD	2.54	2.16	2.89	1.76	1.53	5.61
Susceptible	-	-	-	-	-	-

RR = (LC_{50} or LC_{90} of resistance population / LC_{50} or LC_{90} of susceptible population); HAT = hours after treatment; CSD = central storage depot; LSD = local storage depot

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