

Table of content

Sr. No	Content	Page No.
1	Acknowledgement	i
2	List of Abbreviations	v
3	Introduction	1
4	Chapter 1: Brood morphometry and digging behaviour	19
	1.1 Introduction	19
	1.2 Material and Methodology	25
	1.3 Results	33
	1.4 Discussion	47
	1.5 Conclusion	51
5	Chapter 2: Understanding the nesting behaviour of <i>Digitonthophagus gazella</i>: Role of neurohormones	52
	2.1 Introduction	52
	2.2 Material and Methodology	59
	2.3 Results	65
	2.4 Discussion	73
	2.5 Conclusion	79
6	Chapter 3: Toxic effects of Deltamethrin on biochemical and histological alterations in <i>Digitonthophagus gazella</i>	80
	3.1 Introduction	80
	3.2 Material and Methodology	85
	3.3 Results	93
	3.4 Discussion	118
	3.5 Conclusion	126
7	Chapter 4: Neurophysiological alterations in the nesting behaviour of <i>Digitonthophagus gazella</i> on exposure to Deltamethrin	127
	4.1 Introduction	127
	4.2 Material and Methodology	130
	4.3 Results	137
	4.4 Discussion	157
	4.5 Conclusion	165
8	General Consideration	166
9	Bibliography	184
10	Publication	xx
11	Conferences certificates	xx

List of Table

Table no.	Title	Page No.
Table 1.1	Primers of COI genes obtained	26
Table 1.2	PCR reaction mixture	30
Table 1.3	Reverse transcription cycling program for cDNA synthesis	31
Table 1.4	Real Time PCR mix	31
Table 1.5	Real time PCR conditions	31
Table 1.6	Real time PCR primer sequences of digging genes	31
Table 1.7	Morphological characters for the identification of <i>D. gazella</i>	33
Table 1.8	Observation of tunnel pattern of <i>D. gazella</i> on 10 th , 20 th and 30 th day	37
Table 1.9	Morphological traits of brood balls and shield layers of <i>D. gazella</i>	40
Table 1.10	The developmental period for various stages of life cycle of <i>D. gazella</i>	42
Table 1.11	Brood morphometry of different developmental stages of <i>D. gazella</i>	42
Table 1.12	The fold change in dll and ems in the males and females	45
Table 2.1	PCR reaction mixture	61
Table 2.2	Reverse transcription cycling program for cDNA synthesis	61
Table 2.3	Real Time PCR mix	62
Table 2.4	Real time PCR conditions	62
Table 2.5	Real time PCR primer sequences of neurotransmitter synthesizing enzymes (NTEs)	63
Table 2.6	Real time PCR primer sequences of neuropeptides (NPs)	64
Table 2.7(a)	The level of DA in the brain of <i>D. gazella</i>	66
Table 2.7(b)	The level of 5-HT in the brain of <i>D. gazella</i>	66

Table 2.7(c)	Rate of AChE activity in the brain of <i>D. gazella</i>	67
Table 2.7(d)	The NO content in the brain of <i>D. gazella</i>	68
Table 2.8	The fold change in the expression of ddc and 5-htpdc	69
Table 2.9	The fold change in the level of neuropeptides in male and female <i>D. gazella</i>	71
Table 3.1	Insecticide (Source: PPDB)	81
Table 3.2	PCR reaction mixture	90
Table 3.3	Reverse transcription cycling program for cDNA synthesis	90
Table 3.4	Real Time PCR mix	91
Table 3.5	Real time PCR conditions	91
Table 3.6	Real time PCR primer sequences of neurotransmitter synthesizing enzymes	91
Table 3.7	Probit Mortality obtained after 48 hours of exposure to Deltamethrin	93
Table 3.8	LC ₅₀ value obtained and the sub-lethal doses selected for further studies	93
Table 3.9	Values obtained for the level of SOD, CAT, GSH and LPO after exposure to the sub-lethal concentrations (LD, MD, and HD) of Deltamethrin.	99
Table 3.10	CYP gene expressions after exposure to the sub-lethal concentrations (LD, MD, and HD) of Deltamethrin	104
Table 3.11	Representation of histological alteration in brain of <i>D. gazella</i> on exposure to deltamethrin	112
Table 3.12	Representation of histological alteration in gut region of <i>D. gazella</i> on exposure to deltamethrin	112
Table 3.13	Representation of histological alteration in testis of <i>D. gazella</i> on exposure to deltamethrin	112
Table 1.14	Representation of histological alteration in ovarian follicles of <i>D. gazella</i> on exposure to Deltamethrin	113
Table 4.1	PCR reaction mixture	133
Table 4.2	Reverse transcription cycling program for cDNA synthesis	134

Table 4.3	Real Time PCR mix	134
Table 4.4	Real time PCR conditions	134
Table 4.5	Real time PCR primer sequences of neurotransmitter synthesizing enzymes	135
Table 4.6	Real time PCR primer sequences of neuropeptides	135
Table 4.7	Brain neurotransmitter levels in the male and female <i>D. gazella</i> on exposure to Deltamethrin after (a) 10 (b) 20 and (c) 30 days	138
Table 4.8	Fold change expression of NTEs in the male and female <i>D. gazella</i> , after exposure to Deltamethrin on 10 th , 20 th and 30 th day	145
Table 4.9	Fold change expression of neuropeptides in the male and female <i>D. gazella</i> on exposure to Deltamethrin after 10 th , 20 th , and 30 th day	151

List of Figures

Figure No.	Title	PageNo.
Figure 1.1	Dung beetles classified based on their dung relocation strategies.	21
Figure 1.2	Nesting patterns I, II, and III (Halffter and Edmonds, 1982)	22
Figure 1.3	Collection sites of <i>D. gazella</i> from Vadodara district, Gujarat, India. <i>D. gazella</i> were collected from the outskirts of Vadodara district	25
Figure 1.4	Collection of <i>D. gazella</i> from the selected sites. Burrow opening indicated their presence. The burrow was carefully dug with the help of shovel to collect the beetles.	26
Figure 1.5	Rearing medium for dung beetle. <i>D. gazella</i> were allowed to acclimatize in the convenient size earthen pot placed in the mud tray.	27
Figure 1.6	Observation of tunnel making in rearing medium; arrow points the tunnel formed and the circle represents the branch formed near the blind end of tunnel into which the brood balls are placed	28
Figure 1.7	Morphological features of <i>D. gazella</i>	33
Figure 1.8	Results of Agarose Gel Electrophoresis	34
Figure 1.9	Barcode of COI gene in <i>D. gazella</i>	34
Figure 1.10	Nesting behaviour of <i>D. gazella</i> .	36
Figure 1.11	The tunnel pattern of <i>D. gazella</i> .	38
Figure 1.12	Brood balls formed by <i>D. gazella</i> (a) Spherical shaped brood balls (b) Number of brood balls formed at the end of 10 th , 20 th and 30 th day	39
Figure 1.13	Morphological traits in brood balls formed by <i>D. gazella</i>	40
Figure 1.14	Study on life cycle of <i>D. gazella</i> .	43
Figure 1.15	Comparative account of (a) brood length, (b) brood diameter, and (c) brood weight of different stages of the life cycle of <i>D. gazella</i> .	44
Figure 1.16	The fold change in digging genes in male and female dung beetle (a) dll (b) ems	46

Figure 2.1	Insect brain structure includes mushroom bodies with neurosecretory cells on its periphery and neurpiles inside it	53
Figure 2.2(a)	Dopamine levels on the 10 th , 20 th , and 30 th day of introduction of male and female into the experimental setup	66
Figure 2.2(b)	Serotonin (5-HT) levels on the 10 th , 20 th , and 30 th day of introduction of male and female into the experimental setup	67
Figure 2.2(c)	Rate of AChE activity (with the unit mmol/mL/min x 10 ⁻⁴ per g of tissue) on the 10 th , 20 th , and 30 th day of introduction of male and female into the experimental setup	67
Figure 2.2(d)	Nitric Oxide levels on the 10 th , 20 th , and 30 th day of introduction of male and female into the experimental setup	68
Figure 2.3	Neurotransmitters synthesizing enzyme gene expressions (a) ddc (b) 5-htpdc (b) chat (d) nos	70
Figure 2.4	Fold change expression of neuropeptide gene (a) npf (b) npfr (c) it (d) itr (e) mip in <i>D. gazella</i>	73
Figure 3.1	Dose response curve for the LC ₅₀ determination of Deltamethrin after 48 hours of exposure	93
Figure 3.2a	Fluorescence intensity of the DCFHDA staining, in the single cell suspension of the brain tissue of male <i>D. gazella</i> after exposure to sub-lethal doses (LD, MD, and HD) of Deltamethrin, in comparison to control	94
Figure 3.2b	Fluorescence intensity of the DCFHDA staining, in the single cell suspension of the brain tissue of female <i>D. gazella</i> after exposure to sub-lethal doses (LD, MD, and HD) of Deltamethrin, in comparison to control	95
Figure 3.3	Effects of sub-lethal concentrations of Deltamethrin (LD, MD and HD) in comparison to control, for the generation of ROS in single cell suspension of brain tissue of male <i>D. gazella</i> were determined by DCFH-DA staining (10X,	96

	scale=100µm)	
Figure 3.4	Effects of sub-lethal concentrations of Deltamethrin (LD, MD and HD) in comparison to control, for the generation of ROS in single cell suspension of brain tissue of female <i>D. gazella</i> were determined by DCFH-DA staining (10X, scale=100µm)	97
Figure 3.5	SOD activity after exposure to sub-lethal concentrations of Deltamethrin in comparison to control for 10 th , 20 th and 30 th days	100
Figure 3.6	CAT activity after exposure to sub-lethal concentrations of Deltamethrin in comparison to control for 10 th , 20 th and 30 th day	101
Figure 3.7	GSH levels after exposure to sub-lethal concentrations of Deltamethrin in comparison to control for 10 th , 20 th and 30 th day	102
Figure 3.8	LPO levels after exposure to sub-lethal concentrations of Deltamethrin in comparison to control for 10 th , 20 th and 30 th day	103
Figure 3.9	cyp4q4 mRNA gene expression after exposure to sub-lethal concentrations of Deltamethrin in comparison to control for 10 th , 20 th and 30 th day	105
Figure 3.10	cyp6bq9 mRNA gene expression after exposure to sub-lethal concentrations of Deltamethrin in comparison to control for 10 th , 20 th and 30 th day	106
Figure 3.11	cyp4g7 mRNA gene expression after exposure to sub-lethal concentrations of Deltamethrin in comparison to control for 10 th , 20 th and 30 th day	107
Figure 3.12a	Brain somatic index of <i>D. gazella</i> after exposure to sub-lethal concentrations of Deltamethrin in comparison to control for 10 th , 20 th and 30 th day	108
Figure 3.12b	Gut somatic index of <i>D. gazella</i> after exposure to sub-lethal concentrations of Deltamethrin in comparison to control for 10 th , 20 th and 30 th day	108

Figure 3.12c	Gonad somatic index (male) of <i>D. gazella</i> after exposure to sub-lethal concentrations of Deltamethrin in comparison to control for 10 th , 20 th and 30 th day	109
Figure 3.12d	Gonad somatic index (female) of <i>D. gazella</i> after exposure to sub-lethal concentrations of Deltamethrin in comparison to control for 10 th , 20 th and 30 th day	109
Figure 3.13	Histological sections of <i>D. gazella</i> brain stained with hematoxylin and eosin, after 10 th , 20 th and 30 th day of exposure to Deltamethrin.	114
Figure 3.14	Histological sections of <i>D. gazella</i> midgut stained with hematoxylin and eosin, after 10 th , 20 th and 30 th day of exposure to Deltamethrin.	115
Figure 3.15	Transverse sections of <i>D. gazella</i> testis stained with hematoxylin and eosin, after 10 th , 20 th and 30 th day of exposure to Deltamethrin	116
Figure 3.16	Transverse sections of <i>D. gazella</i> ovaries stained with hematoxylin and eosin, after 10 th , 20 th and 30 th day of exposure to Deltamethrin	117
Figure 4.1	The association of neuroendocrine regulation in the nesting behaviour of <i>D. gazella</i> on exposure to Deltamethrin	130
Figure 4.2	Number of brood balls formed by <i>D. gazella</i> after 10 th , 20 th and 30 th day of exposure to Deltamethrin	137
Figure 4.3	Brain DA levels in the male and female <i>D. gazella</i> on exposure to Deltamethrin after (a) 10 days (b) 20 days and (c) 30 days	140
Figure 4.4	Brain 5-HT levels in the male and female <i>D. gazella</i> on exposure to Deltamethrin after (a) 10 days (b) 20 days and (c) 30 days	141
Figure 4.5	Brain AChE activity in the male and female <i>D. gazella</i> on exposure to Deltamethrin after (a) 10 days (b) 20 days and (c) 30 days	142
Figure 4.6	Brain NO levels in the male and female <i>D. gazella</i> on exposure to Deltamethrin after (a) 10 days (b) 20 days and	143

	(c) 30 days	
Figure 4.7	ddc fold change in the brain of male and female <i>D. gazella</i> on exposure to Deltamethrin after (a) 10 days (b) 20 days and (c) 30 days	146
Figure 4.8	5-htpdc fold change in the brain of male and female <i>D. gazella</i> on exposure to Deltamethrin after (a) 10 days (b) 20 days and (c) 30 days	147
Figure 4.9	chAt fold change in the brain of male and female <i>D. gazella</i> on exposure to Deltamethrin after (a) 10 days (b) 20 days and (c) 30 days	148
Figure 4.10	nos fold change in the brain of male and female <i>D. gazella</i> on exposure to Deltamethrin after (a) 10 days (b) 20 days and (c) 30 days	149
Figure 4.11	npf fold change in the brain of male and female <i>D. gazella</i> on exposure to Deltamethrin after (a) 10 days (b) 20 days and (c) 30 days	152
Figure 4.12	npfr fold change in the brain of male and female <i>D. gazella</i> on exposure to Deltamethrin after (a) 10 days (b) 20 days and (c) 30 days	153
Figure 4.13	it fold change in the brain of male and female <i>D. gazella</i> on exposure to Deltamethrin after (a) 10 days (b) 20 days and (c) 30 days	154
Figure 4.14	itr fold change in the brain of male and female <i>D. gazella</i> on exposure to Deltamethrin after (a) 10 days (b) 20 days and (c) 30 days	155
Figure 4.15	mip fold change in the brain of male and female <i>D. gazella</i> on exposure to Deltamethrin after (a) 10 days (b) 20 days and (c) 30 days	156
