GENERAL CONSIDERATION

Chapter 1: Brood morphometry and digging behaviour

Scarabaeinae, commonly known as dung beetles, have a close evolutionary relationship and primarily feed and reproduce on organic materials like dung, carrion, and decaying fruits (Scholtz et al., 2009; Simmons and Ridsdill-Smith, 2011). They serve various ecological functions and exhibit diverse morphological and behavioural adaptations, making them globally distributed. Dung beetles aid in dung decomposition by utilizing it as a source of fibrous material for their larvae (Arenallo et al., 2017). Their preference for dung type varies, with a preference for omnivorous dung over herbivorous and carnivore dung (Frank et al., 2017; Pandya et al., 2023). Dung beetle behaviour, including dung consumption and relocation, is influenced by factors like soil type, moisture, dung quality, and pair cooperation (Nichols et al., 2008; Braga et al., 2013; Slade et al., 2011; De Groot et al., 2002; Banerjee, 2014; Tarasov and Dimitrov, 2016; Singh et al., 2019). Dung beetles are categorized into four groups based on their food resource utilization and nest-building habits: Telecoprids (rollers), which roll dung away before burying it; Endocoprids (burrowers), which lay eggs directly within the dung; Kleptocoprids (dwellers), which lay eggs in dung buried by other dung beetles; and Paracoprids (tunnelers), which construct nests beneath the dung pad before laying eggs (Halffter and Matthews, 1966; Doube, 1990; Hernández et al., 2011; Chao et al., 2013). Additionally, dung beetles contribute to soil fertility, permeability, plant growth, seed dispersal, and the control of parasitic growth while reducing greenhouse gas emissions through dung utilization (Latha and Sabu, 2018).

Despite their ecological importance, dung beetle diversity is declining due to human activities in forests and pastures (Nichols et al., 2009; Basto-Estrella et al., 2014; Kim et al., 2021), posing a threat to their populations and the ecosystem services they provide (Nichols et al., 2008). Therefore, it is crucial to conduct in-depth studies on dung beetles to ensure the health of ecosystems (Salomão et al., 2020).In the realm of insects, parental care takes on various forms (Tallamy and Wood, 1986; Clutton-Brock, 1991; Trumbo, 2012). Some species exhibit pre-oviposition care, involving actions like provisioning food masses, selecting suitable egg-laying sites, constructing nests, providing protective structures or chemical defences for eggs and larvae, and modifying environmental conditions (Royle et al., 2012; Smiseth et al., 2012; Machado and Trumbo, 2018).

Dung beetles, in particular, invest substantial time and energy in nest construction and brood protection. Typically, they deposit a single egg into an egg chamber and seal it (Hunt and Simmons, 2000). The larva remains inside this chamber until it pupates, emphasizing the critical role of the brood ball's integrity for survival during development. Some brood balls incorporate a narrow aeration conduit that connects the outside environment to the egg chamber, sometimes featuring a filter made from dung fibers (Cantil et al., 2014). The utilization of nutrient-rich yet fleeting dung resources by growing offspring prompts distinctive behavioural and physiological adaptations, fostering sub-sociality and biparental care (Arce et al., 2012; Panaitof et al., 2016; Heurta et al., 2013). This care offers protection to offspring against competition and desiccation (Rauter and Moore, 2002; Kim et al., 2021). Biparental care is prevalent in dung beetles of the *Onthophagus* genus, characterized by a branched tunneling system (Hal€ter and Edmonds, 1982). Additionally, there is evidence suggesting that parental provisioning strategies may vary with parental size, as observed in *D. gazella*, where larger parents produce larger brood masses (Hunt and Simmons, 2002; Steiger, 2013). Although significant knowledge exists regarding tunnelling dung beetles, few studies have delved into the nesting and reproductive behaviour of ball-rolling dung beetles, with a particular focus on *D. gazella*. Therefore, this study was focused *D. gazella's* nest architecture, brood morphometry, and the digging behaviour.

Digitonthophagus gazella, known for its dung-rolling and nesting behaviour, shares nesting traits with other *Onthophagus* species (Huerta and García-Hernández, 2013; Arellano et al., 2017; Sane et al., 2020). However, in our observations, some differences were noted which included cooperation between sexes during ball rolling and initial nest construction stages. The nesting pattern observed in the early stages was identified as a type II pattern, consistent with tunneler dung beetles, aligning with previous findings (Heurta et al., 2023). Furthermore, the study revealed time-dependent complexity in tunnel formation. By the 10th day, the tunnel had three branches, which increased to four branches by the 20th and 30th day, housing linearly arranged brood masses. This observed increase in tunnel length and total depth over time corresponds to findings by Sane et al. (2020), suggesting a Markovian-building process in dung beetles. This process allows them to construct larger, deeper pits with steeper walls for brood protection, and no variation in tunnel diameter among similar-sized dung beetles, contrary to previous reports linking tunnel width to beetle body size (Klingenberg and Monteiro, 2005; Bertossa, 2011; Macagno et al., 2016). Further, in line with these previous findings, the present study on *D. gazella* in laboratory conditions revealed that cooperation between males and females led to the excavation of deeper tunnels and the production of an increased number of brood balls, ranging from a mean of 50 to 155 over a period of 10 to 30 days of nest construction (Arenallo et al., 2017; Johari et al., 2023; Kerman et al., 2023).

In our study, we observed the life cycle of *D. gazella* to be approximately 28 to 30 days, consisting of four distinct stages: egg, larva (3 instars), pupa, and adult. This developmental period likely correlates with the controlled ambient temperature maintained during the experiment and aligns with the timelines observed in other Scarabaeinae species, such as 34-38 days in *O. incensus*, 39 days in *O. lecontei*, 30-34 days in *Nesosisyphus spp*., 30-35 days in *O. reticornustus*, and 30 days in *O. taurus*. Throughout the life cycle, we noted significant changes in the morphometry of the developing brood, including length (from 2.49±0.08 mm to 14.67±1.78 mm), diameter (from 1.47±0.09 mm to 6.9±1.49 mm), and weight (ranging from 6 mg to 139 mg). Interestingly, there was no variation in the length of the brood ball, consistent with findings by Arellano et al., (2017) and Singh et al., (2019). Upon reaching adulthood, dung beetles emerge from the brood ball and undergo sexual maturation (Huerta and García-Hernández, 2013). Subsequently, they engage in nesting activities, which include digging tunnels, constructing brood balls, mating, and female egg-laying. Dung beetles possess three pairs of legs with distinct leg segments, including the femur, tibia, and tarsal segments (Linz et al., 2019). Dung rollers have a powerful digging apparatus on their fore tibia, featuring teeth for excavating compacted soil, and a tibial spur at the distal end, crucial for digging. The shovel-like structure of the tibia is believed to influence various activities such as reproduction, competition, and cooperation, all of which significantly impact ecosystem functions and services, leading to the evolution of tunnelling and subterranean reproduction as unique life-history strategies (Fernandes et al., 2011; Nervo et al., 2022). Previous studies combining behavioural and developmental genetic approaches have highlighted the functional enhancement of digging performance by tibial teeth and the involvement of diverse genes and pathways in tibial teeth formation (Linz et al., 2019).

Based on the analysis of 16 leg genes, 13 are known to be essential for proper tibial teeth formation, while 7 genes are associated with leg patterning, including tibia formation. Additionally, 6 genes have roles in leg formation, and 2 genes uniquely affect tibial teeth size, shape, and spacing (Angelini et al., 2012). In our study, we employed a combination of behavioural and genetic approaches to assess the significance of the front tibia, specifically focusing on the expression of digging genes (dll and ems). A significant ($p<0.5$) decrease in dll and ems expression, observed over time from 10 to 30 days in both males and females, underscores the functional importance of these digging genes in the initial phase of nesting behavior. Our findings align with previous work by Linz et al., (2019) and Jugovic and Koprivnikar, (2021), emphasizing the pivotal role of tibial genes in the behaviour and ecology of dung beetles.

Thus, the present study provides valuable insights into the nesting behaviour of *D. gazella,* which was examined under controlled laboratory conditions. It places a specific emphasis on key aspects of their nesting behaviour, including tunnelling, brood ball construction, and parental care. This investigation is particularly significant because, despite extensive research into the costs and benefits associated with biparental cooperation in various species, there remains a knowledge gap regarding the intricate neurophysiological and molecular mechanisms responsible for driving plasticity in nesting behaviour. Neuromodulation refers to the regulation of neural activities and behaviours through the modulation of neurotransmitter systems and other neural mechanisms. Understanding how neuromodulation influences nesting behaviours is crucial for unravelling the precise neural and physiological mechanisms that enable *D. gazella* to adapt and exhibit flexible nesting behaviour. Further research in chapter 2 thus paves the way for more investigations that can delve into the intricate neural and molecular mechanisms underlying the remarkable flexibility in nesting behaviours observed in dung beetles. Such studies can have broader implications not only for our comprehension of insect behaviour but also for the broader field of behavioural ecology and neurobiology.

In conclusion, this study serves as a crucial stepping stone in the exploration of the nesting behaviors of *D. gazella* dung beetles, shedding light on their plasticity and emphasizing the need for further research into the neuromodulatory aspects that drive these flexible behaviors. It is a testament to the ongoing pursuit of knowledge about the intricacies of the natural world and the diverse mechanisms that enable organisms to adapt and thrive in ever-changing environments.

Chapter 2: Understanding the nesting behaviour of D. gazella: role of neurohormones

Dung beetles exhibit behaviours such as dung ball rolling and tunneling, vital for survival and reproduction, including parental care (Moczek, 2009; Khadakkar et al., 2019). These behaviours are underpinned by complex physiological processes, including neurotransmitter activity, such as DA, 5-HT, ACh, octopamine, and GABA (Verlinden et al., 2010; Kannan et al., 2022; Xing et al., 2023), which play essential roles in insect behaviour. Biogenic amines, such as 5-HT and DA, are key neuroactive substances that modulate neural responses, muscle movements, and behaviour (Watanabe and Sasaki, 2021; Sasaki and Watanabe, 2022). These amines activate neural circuits to regulate behaviour (Libersat and Pflueger, 2004; Bergan, 2015). Diverse aminergic circuits and receptor expression patterns across insect species result in varied activities (Barron et al., 2010; Perry et al., 2016; Blenau and Thamm, 2011). DA and 5-HT interact with hormone signalling pathways to elicit distinct behavioural and developmental responses (Pfaff and Joels, 2016). Dung beetles, including *D. gazella*, exhibit familial sociality, such as biparental care (Cunningham et al., 2015; Panaitof et al., 2016). These beetles have intricate neuroendocrine control mechanisms related to their reproductive strategies, including tunnelling and parental care (Hunt and Simmons, 2002; Harano et al., 2008).

Neurotransmitter-synthesizing enzymes, including DDC, 5-HTPDC, ChAT, and NOS, validate the activity of these neurotransmitters (Vavricka, 2014; Hiragaki, 2015). For instance, DDC is responsible for dopamine synthesis, associated with parental care and aggression (Lonstein, 2002; Zhao and Li, 2009). 5-HTPDC synthesizes serotonin, linked to sociality, parental care, and aggression (Antsey et al., 2009; Dulac et al., 2014). ChAT produces acetylcholine, crucial for olfactory learning and memory in insects (Grünewald and Siefert, 2019), while NOS generates nitric oxide, regulating various physiological processes, feeding, mating, and aggression (Bicker, 2000; Rillich and Stevenson, 2019).

Neuropeptides, like NPF and inotocin, also play significant roles in regulating insect physiology and behaviour (Pedrazzini et al., 2003; Yue et al., 2017; Debiec, 2005; Lim and Young, 2006; Gruber et al., 2012; Johnson et al., 2017). Additionally, the Wamide neuropeptide superfamily is involved in life cycle transitions and larval settlement (Liu et al., 2008; Kim et al., 2006a; Kim et al., 2006b; Santos et al., 2007; Davis, 2003). Myosin Inhibiting Peptide (MIP) regulates muscle contraction and influences reproductive behaviours

(Lange et al., 2012; Hasebe and Shiga, 2021). Studying these neurochemical aspects provides insights into the molecular mechanisms behind the complex behaviours and adaptations observed in dung beetles like *D. gazella*. This understanding can contribute to a more comprehensive comprehension of the relationship between behaviour and physiology in these fascinating insects.

In the present study, elevated levels of DA and 5-HT on the 10^{th}, 20^{th}, and 30^{th} days of tunneling suggest the involvement of these neurotransmitters in nesting behavior (Misof et al., 2014; Song et al., 2015; Kamhi et al., 2017). Males exhibited a slightly higher 5-HT titer than females, potentially linked to context-dependent male aggression during copulation (Trumbo, 2019). The increased levels of DA in both males and females further support its role in reinforcing nesting activities. These findings align with previous research (Auletta, 2019) highlighting the critical roles of NTs in various behaviours and physiological processes. NTs modulate complex social activities, affecting reproductive strategies (Kamhi et al., 2017; Sasaki and Watanabe, 2022).

Mating triggers changes in behaviour and physiology, including increased oviposition and re-mating, mediated by peptides and proteins from male accessory glands (Carmel et al., 2016) and physical stimulation (Li et al., 2020). The elevated levels of DA in response to olfactory stimuli and mating during nesting are apparent in the present study. The study also explored acetylcholinesterase (AChE) levels, which are associated with physical activity in insects (Hao et al., 2021). AChE levels were higher in females than males, potentially due to the increased physical activity of females during tunnel making and brood ball formation (Nervo et al., 2022). Nitric oxide (NO) was implicated in various physiological processes and behaviours, including reproduction, learning, and memory (Strauss, 2002; Popov et al., 2005; Wessnitzer and Webb, 2006). NO likely plays a role in signalling mechanisms, influencing foraging behaviour and other complex activities in insects (Koto et al., 2019).

Neurotransmitter biosynthesis enzymes, such as DDC and 5-HTPDC, are critical for the formation of DA and 5-HT (Farooqi et al., 2022). These enzymes' gene expression paralleled the increase in NT levels, confirming their involvement in *D. gazella*'s nesting behaviour. Choline Acetyl Transferase (ChAt) and acetylcholinesterase (AChE) are linked to olfactory learning, muscle control, and sensory processing. The study's findings highlight the role of these enzymes in dung location, navigation, and reproductive strategies.

Further, neuropeptides like npf, it, and mip were found to influence various aspects of reproductive behaviour, aggression, feeding, and motor control (Nässel and Homberg, 2006; Potticary et al., 2022; Hussain et al., 2016). Inotocin, in particular, likely plays a role in parental care and reproductive behaviour, as its levels increased during nesting. Myosin inhibiting peptide was implicated in muscle activity, feeding, and oviduct contractions (Hensgen et al., 2022). Its increased expression in the present study, likely helps coordinate complex behaviours, such as dung ball rolling and foraging, by regulating muscle contractions in *D. gazella*.

In summary, the study has unveiled a complex web of interactions among neurotransmitters, enzymatic processes, and neuropeptides that intricately regulate the nesting behaviour of *D. gazella*. These findings offer profound insights into the neural mechanisms that underpin the intricate behaviours observed in these insects and may have broader implications for understanding complex behaviours in other insect species. The study's findings highlight the pivotal role of neurotransmitters in orchestrating and modulating nesting behaviours. Neurotransmitters are instrumental in coordinating the precise sequence of actions required for successful nesting, from tunnel excavation to brood mass construction. Moreover, the study has illuminated the significance of enzymatic processes in the regulation of nesting behaviour. Enzymes are the molecular catalysts that facilitate biochemical reactions within an organism. In the context of this study, they are crucial for the synthesis and degradation of neurotransmitters and neuropeptides. This dynamic enzymatic activity influences the availability and balance of these critical signalling molecules, directly impacting the finely tuned nesting behaviours exhibited by *D. gazella*. Perhaps most intriguingly, the research has brought attention to the role of neuropeptides in modulating nesting behaviour. Neuropeptides are bioactive molecules that act as neuromodulators and can significantly influence an insect's behaviour and physiology. The study's findings suggest that these neuropeptides are intimately involved in shaping the nesting behaviours of *D. gazella* dung beetles. They likely serve as regulators and coordinators of the intricate actions and sequences required for nesting success. Importantly, these insights into the neural mechanisms underlying complex behaviours in dung beetles may extend to other insect species such as those involved in foraging, mating, and territoriality, and are integral to their survival and reproduction. Understanding the neural basis of these behaviours in *D. gazella*, provides a foundation for exploring similar mechanisms in other insects, enriching our understanding of their behaviour as a whole.

In conclusion, this study's revelations about the interplay between neurotransmitters, enzymatic processes, and neuropeptides in regulating nesting behaviour in *D. gazella* offer a deeper appreciation for the intricacies of insect behaviour. These findings contribute to the broader field of insect neuroscience and have the potential to inform future research aimed at unravelling the neural underpinnings of complex reproductive behaviours.

Chapter 3: Toxic effects of Deltamethrin on biochemical and histological alterations in D. gazella

Deltamethrin, a synthetic pyrethroid (Meunier et al., 2020), is widely known for its impact on sodium channels. It is extensively used and even sub lethal concentrations cause significant harm to both target and non-target insects (Cutler, 2013; Müller, 2018). Deltamethrin induces oxidative stress, resulting in the production of reactive oxygen species (ROS), including superoxide radicals (O_{2-}), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH-), which are highly reactive and damage various cellular components (Zug and Hammerstein, 2015; Hattab et al., 2015). Several classes of insecticides, including pyrethroids like Deltamethrin, organophosphates, and neonicotinoids, can induce ROS production (Krůček et al., 2015). ROS disrupt essential cellular functions, decrease reproductive performance, and lead to cell damage or dysfunction (Nancy et al., 2021; Blount et al., 2016). Additionally, ROS can affect the cell membrane's electrochemical gradient (Chen et al., 2010; Faize et al., 2011).

Insects possess natural antioxidant response systems, including low-molecular antioxidants and antioxidant enzymes that detoxify ROS and protect biomolecules (Halliwell and Gutteridge, 2015). These systems are vital for maintaining homeostasis and participating in cell-signaling pathways, programmed cell death, gene expression, and immune defense (Gupta et al., 2010; Świętek et al., 2019). Antioxidative mechanisms combat oxidative stress, and the activity of enzymes like superoxide dismutase (SOD), catalase (CAT), and glutathione-S-transferases (GSTs) plays a role in this defence (Dey and De, 2012). Inhibition of these antioxidant enzymes can further elevate ROS levels (Yildiztekin et al., 2015; Singh and Kaur, 2016). Further, cytochrome P450 (CYP450) enzymes, found in insects, are crucial for metabolizing insecticides and xenobiotics (Suchail et al., 2004; Liu and Zhang, 2004). They are organized into families like CYP4, CYP6, CYP9, CYP12, and CYP345 (Mao et al., 2009). In some scarab beetle taxa, including pest species, CYP450 induction is a critical mechanism for neonicotinoid detoxification (Cavallaro et al., 2022; Adesanya et al., 2018).

Furthermore, Deltamethrin also influences the shape and structure of internal organs and tissues in various organisms. Histopathological changes in insects can serve as biomarkers for assessing health following exposure to chemicals. These changes are observed as alterations in tissue integrity and are associated with complex biochemical and physiological responses to stressors (Deveci et al., 2021). Various insecticides, including spinosad, imidacloprid, permethrin, have been reported to induce histological, physiological, and behavioural impacts in insects (Cisneros et al., 2002; Lakshmi et al., 2010; Martelli et al., 2020; El-Ashram et al., 2021; Martínez et al., 2018; Vinha et al., 2021).

Deltamethrin mode of action, involving contact and ingestion, primarily affects the brain and gut, which can consequently influence reproductive physiology in insects. However, there is limited research linking oxidative stress, histological changes, and their effects on insects, particularly *D. gazella*. This study was focused on the comprehensive examination of toxic potential of Deltamethrin, focusing on ROS-dependent neurotoxicity and its impact on the histological structure of the brain, gut, and gonads in *D. gazella*.

Exposure to sub lethal doses of Deltamethrin is known to influence various insect behaviours, but its effects on *D. gazella* remain unexplored. In our study, we investigated the toxic potential of Deltamethrin at sub-acute doses (LD, MD, and HD) relative to its LC_{50} value (0.275 ppm at 48 hours) in *D. gazella*, a commonly used pesticide in pasturelands. Deltamethrin exposure resulted in jerky movements, loss of equilibrium, tremors, and integument darkening, possibly entering through contact and ingestion. These behavioural changes are attributed to its interference with voltage-gated sodium channels in insect neuronal membranes, disrupting nervous system signalling.

Deltamethrin is known to cause toxicity in vertebrates and invertebrates due to oxidative stress, reactive oxygen species (ROS) generation, and altered metabolism. In this study, DCFH-DA staining confirmed a dose and time-dependent increase in ROS production in the brain tissue. Oxidative stress induced by ROS is implicated in the toxicity of many pesticides. The study also examined the response of *D. gazella* to oxidative stress through quantitative analysis of lipid peroxidation (LPO) and antioxidant mechanisms (SOD, CAT, GSH). The depletion of GSH levels suggested an impaired antioxidant defence. Additionally, cytochrome P450 (CYP450) activity, involved in detoxification of toxicants in insects, was assessed. Deltamethrin exposure led to a significant down regulation of CYP4G7, CYP6BQ9, and CYP4Q4 expression in a dose and time-dependent manner. This down regulation contrasts with previous studies that found CYP6BQ9 to be predominantly expressed in the brain of pyrethroid resistant insects. The study didn't focus on insecticide resistance but highlighted the impact of Deltamethrin on CYP450 activity.

We also performed the organosomatic indices and histopathological examinations to assess the effects of Deltamethrin on the brain, gut, and gonads of *D. gazella*. In the gut, there was a dose and time-dependent decrease in the gut somatic index, accompanied by histological alterations suggesting reduced digestion and absorption, impacting energy production and overall physiology. In the gonads, both ovary and testis showed reduced gonadosomatic indices and histomorphological changes indicative of disrupted reproductive potential.

In summary, the exposure of Deltamethrin has been found to have far-reaching and irreversible pathological consequences on various vital organs and systems within *D. gazella*, including the brain, midgut, and gonads. These findings underscore the considerable risks associated with Deltamethrin exposure for non-target dung beetles, which hold significant ecological and economic importance. The profound pathological effects observed in the brain of *D. gazella* indicate that Deltamethrin disrupts critical neurological processes. This can lead to altered behaviour, impaired sensory perception, and compromised motor skills, ultimately jeopardizing the beetles' ability to perform essential functions such as nesting, foraging, and reproduction. Secondly, the detrimental effects on the midgut are of great significance. The midgut is responsible for digestion and nutrient absorption, which are pivotal for the beetles' survival and reproductive success. Deltamethrin-induced damage to the midgut can disrupt these vital processes, leading to malnutrition and reduced energy reserves. This, in turn, can compromise the beetles' overall health and fitness, making them more susceptible to environmental stressors and reducing their reproductive potential. Lastly, the observed impacts on the gonads are particularly concerning in the context of population sustainability. The gonads are responsible for reproduction, and any damage or dysfunction in these organs can directly affect the beetles' ability to reproduce successfully. Irreversible pathological changes in the gonads can lead to reduced fertility, altered mating behaviours, and, over time, a decline in the population of *D. gazella*. It's important to emphasize that dung beetles like *D. gazella* play critical roles in ecosystems and agriculture. They facilitate nutrient cycling, improve soil health, and help control pests, making them essential contributors to both ecological balance and economic productivity. Consequently, the potential risks posed by Deltamethrin exposure to these non-target beetles have broader implications for ecosystem stability and agricultural sustainability.

In conclusion, the pathological effects of Deltamethrin exposure on the brain, midgut, and gonads of *D. gazella* underscore the need for careful consideration of the environmental impact of pesticides on non-target species. These findings emphasize the importance of adopting sustainable and ecologically responsible pest management practices to safeguard the invaluable services provided by dung beetles in our ecosystems and agricultural systems.

Chapter 4: Neurophysiological alterations in the nesting behaviour of D. gazella on exposure to Deltamethrin

Given their pivotal roles in physiological and behavioural processes, impacting an organism's survival in challenging environments, it is reasonable to anticipate that, in response to insecticide exposure, molecules governing neural regulation in the nesting behaviour of dung rollers, such as neurotransmitters and neuropeptides, could play significant roles. Current scientific knowledge has established that biogenic amines, notably octopamine (OA), dopamine (DA), and serotonin (5-HT), are implicated in the insect stress response (Gruntenko et al., 2016). Moreover, the levels of these biogenic amines have been observed to fluctuate in various insect species under adverse conditions (Hirashima et al., 2000; Chentsova et al., 2002). Biogenic amines exhibit a wide spectrum of actions, including the regulation of behaviours like feeding, reproduction, and social interactions (Pflüger and Duch, 2011). Importantly, they evoke systemic responses to various environmental factors, including stressors like insecticides (Adamo, 2008). For instance, research has demonstrated that following exposure, OA and DA are rapidly released into an insect's hemolymph, initiating a cascade of reactions aimed at restoring homeostasis (Gruntenko et al., 2004). Intriguingly, the release of these biogenic amines during stress does not seem to be specific to the type of stressor (Armstrong and Robertson, 2006; Gruntenko and Rauschenbach, 2018). Additionally, pyrethroids have been shown to alter neurotransmitter levels and metabolites of monoamine neurotransmitters in the insect brain (Kori et al., 2018).

Neuropeptides (NPs) also hold a central role in regulating various physiological and behavioural processes in insects, encompassing reproduction, development, growth, metabolic homeostasis, longevity, and stress responses (Lubawy et al., 2020). In beetles, the synthesis and release of multiple NPs are intricately controlled (Yeoh et al., 2017). This system is tightly regulated and appears susceptible to unfavourable conditions like low temperature, cold conditions, or stress induced by insecticides (Li et al., 2020). Under stress, various chemical signals are released, either through direct intercellular contact or systemically. These signals govern behavioural responses, including neuropeptide-like peptides such as npf, it, and mip, which are involved in the stress response and potentially contribute to its regulation (Schoofs et al., 2017; Ragionieri et al., 2022).

Studies employing molecular genetics techniques have delved into the functional roles of neuropeptides and their receptors in *D. melanogaster* and the cockroach *Leuco phaeamaderae* (Nässel and Homberg, 2006). Nässel and Zandawala, (2019) highlighted novel neuropeptides and advanced genetic techniques, unveiling how peptides act within CNS circuits to modulate behavior and physiology. Ragionieri et al. (2022) confirmed the conservation of neuropeptide genes in *Schistocerca*, while Pandit et al., (2018) identified neuropeptide F in the model beetle *T. castaneum* and explored numerous neuropeptide-related transcripts in *H. abietis*. Furthermore, Ragionieri and Predel, (2020) extensively investigated putative neuropeptide precursors in the carabid beetle *Pogonus chalceus*.

Insecticide exposure can disrupt the chemical communication system, resulting in changes in various insect behaviours, including food foraging, oviposition site selection, and pheromonal communication. These changes involve complex physiological mechanisms driven by hormones and neurohormones, ultimately reducing the insects' reproductive success due to behavioural changes, particularly during the reproductive phase (França et al., 2017). Some insecticides targeting the endocrine system may also influence reproductive behaviour, as seen with Deltamethrin's effects on the calling behaviour and sex pheromone production in *Ostrinia furnacalis* (Wei et al., 2004). Thus, present study confirmed the toxic potential of Deltamethrin on biochemical and histological parameters and shed light on its involvement in altered neurophysiology by investigating the role of neurotransmitters (biogenic amines and nitric oxide) and neuropeptides in the nesting behaviour of *D. gazella*.

Deltamethrin is a widely used synthetic pyrethroid insecticide known to cause physiological damage and reduced reproductive potential in various insect species (Cutler, 2013; Rehman et al., 2014; Müller, 2018). In *D. gazella*, Deltamethrin exposure reduced the number of brood balls over time, indicating a loss of functional efficiency and reduced reproductive potential which further lead to behavioural alterations, including changes in neurotransmitter levels, particularly the inhibition of acetylcholinesterase (AChE). AChE inhibition prevents the breakdown of acetylcholine, leading to prolonged nerve cell depolarization. Additionally, the interaction between AChE and choline acetyltransferase (ChAT) may play a role in insecticide exposure (Grünewald and Siefert, 2019; Bourguet et al., 2020; Johnson et al., 2021).

Deltamethrin exposure induced oxidative stress and affects dopamine neurotransmission, altered gene expression of dopamine synthesizing enzymes (ddc), which might lead to changes in locomotion and other behaviours (Figueira et al., 2017; Xu et al., 2015). This disruption in dopamine transmission can affect the nesting behaviour of *D. gazella*, including tunneling, reproduction, brood ball formation, and parental care (França et al., 2017). Furthermore, a significant reduction in 5-HT levels and the 5-HT synthesizing enzyme (5-htpdc) was observed in *D. gazella*, suggesting a role for 5-HT in modulating nesting behaviours (Dillen et al., 2013; Deng et al., 2014). Nitric oxide (NO) is another important signaling molecule in insects, associated with various physiological processes (Eleftherianos et al., 2009; Ishii et al, 2013.). Deltamethrin exposure reduced the NO levels and expression of nitric oxide synthase (NOS), indicating its involvement in altering nesting behaviour of *D. gazella* (Sadekuzzaman et al., 2018).

Additionally, neuropeptides (NPs), such as neuropeptide F (npf), inotocin (it), and myoinhibiting peptides (mip), have critical roles in regulating physiological functions in insects, including feeding, metabolism, and reproduction (Yeoh et al., 2017; Liutkeviciute et al., 2016). Deltamethrin exposure led to a dose-dependent reduction in the expression of these neuropeptides (npf, it, and mip) and their receptors (npfr, itr), suggesting their involvement in modulating nesting behaviour (Aljedani, 2021; Sakthivel et al., 2022).

In conclusion, the exposure of Deltamethrin has been shown to have a significant impact on the nesting behaviour of *D. gazella*. This impact is not a singular event but rather the result of a cascade of interconnected mechanisms that influence the neurological and behavioural aspects of these dung beetles. Specifically, it disrupts the delicate balance of neurotransmitters like dopamine and serotonin, which are crucial for regulating various aspects of nesting behaviour, including tunnelling, brood ball making, reproduction, and brood care. Furthermore, the pesticide has been found to modulate the activity of neuropeptides and their corresponding receptors. Neuropeptides are essential regulators of a wide range of physiological processes, including those related to behaviour. The alteration of these neuropeptide systems can have profound effects on the behaviour of *D. gazella*. These intricate and interrelated mechanisms result in a spectrum of neurological manifestations within the beetles, ultimately impacting their reproductive efficiency and overall behaviours. The nesting behaviour, which is crucial for their reproductive success and survival, is particularly vulnerable to these disruptions.

Overall Conclusion

The present study significantly advances our understanding of the nesting biology and neural regulation in *D. gazella*, a crucial species in various ecosystems. The study delves into several key aspects of their behaviour and physiology, shedding light on how they construct nests, reproduce, and navigate their environment.

One of the fundamental contributions of this research is the revelation of *D. gazella*'s nesting behaviour. Both males and females play active roles in tunnel construction and the preparation of brood balls, which are essential for their reproductive success. The study provides a detailed account of the nest structure, including the type II pattern, and the timeline of nest-building activities within 10^{th}, 20^{th}, and 30^{th} day. Additionally, it highlights the time-dependent changes in the number and morphometry of brood balls, illustrating the species' unique nesting characteristics. Furthermore, the study proved the genetic underpinnings of nesting behaviour by examining the expression of digging genes (dll and ems) and was observed to increase on 10^{th} day when they covered the maximum length of tunnel, suggesting their crucial role in the nest-building process, marking a significant finding in our understanding of dung beetle behaviour.

The research also delves into the intricate neural mechanisms that govern these behaviours. The study confirms the involvement of neurotransmitters such as dopamine (DA), serotonin (5-HT), acetyl cholinesterase (AChE), and nitric oxide (NO) in regulating critical aspects of nesting behaviour, including digging, brood ball formation, navigation, and reproduction. The increased activity of neurotransmitter biosynthesizing enzymes provides solid evidence of their role in mediating these behaviours. Additionally, our study has also validated the neuropeptides (npf, it, and mip) gene expressions as key influencers of various behavioural and physiological processes, further enriching our understanding of the neural regulation of *D. gazella*'s nesting behaviour.

A crucial aspect illuminated by this research is the adverse impact of Deltamethrin exposure on *D. gazella*. The commonly used insecticide is shown to disrupt the subtle balance of antioxidants, leading to decreased enzyme activity and an increase in harmful reactive oxygen species (ROS). This oxidative stress, coupled with tissue damage, inflammation, and structural abnormalities in vital organs such as the brain, gut, ovaries, and testes, underscores the toxic potential of Deltamethrin. These findings emphasize the need for responsible pesticide use and the importance of monitoring for signs of toxicity in exposed organisms.

In conclusion, this study offers a comprehensive exploration of *D. gazella*'s nesting biology and the neural regulation of their behaviour. It underscores the profound impact of Deltamethrin exposure on their physiology and behaviour, highlighting the potential risks associated with pesticide use in ecosystems where dung beetles are pivotal. This research serves as a valuable resource for further investigations into the conservation and management of these ecologically significant insects while emphasizing the importance of environmentally responsible pest control practices.

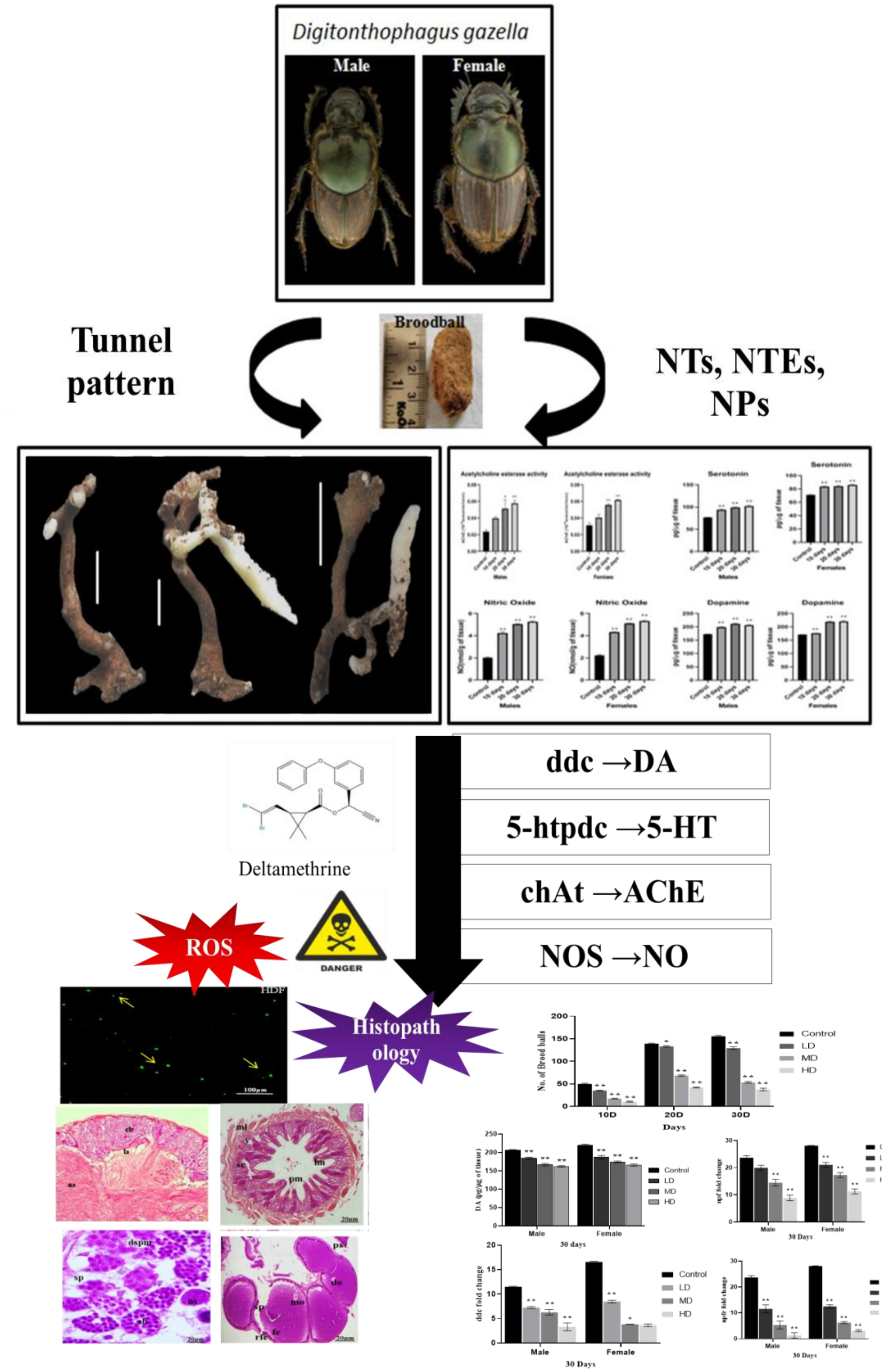

Figure GCI: Graphical Abstract of the neural regulation in the nesting behaviour of *D. gazella* on exposure to Deltamethrin

Future Prospects and Recommendations

1. The present study has identified alterations in nesting behaviours and gene expression related to neuropeptides and neurotransmitters in *D. gazella*. To validate these findings, Western blotting can be employed to quantify protein expression levels. Western blotting will allow us to confirm whether the changes in gene expression translate into changes in protein abundance.
2. Immunohistochemistry can be performed to precisely locate and understand the regulation of neurotransmitters within the brain. This technique can provide spatial information on neurotransmitter distribution.
3. Next-generation sequencing (NGS) holds the potential to enhance the authentication of *D. gazella* and uncover more about its genome characteristics. NGS can also shed light on the toxicity mechanisms of Deltamethrin at the genetic level. By analysing gene expression profiles and identifying key genes affected by exposure to Deltamethrin, we can gain insights into the molecular response of *D. gazella* to this insecticide.
4. Comparing the tunnelling patterns of *D. gazella* with those of other dung beetles using electron microscopy can reveal species-specific adaptations and ecological implications. In addition to histology; electron microscopy can provide high-resolution images of tissue, cell, and organelle structures. This advanced imaging technique will allow for a detailed examination of the structural and microarchitecture changes induced by Deltamethrin exposure.
5. Epigenetic modifications, such as DNA methylation and histone modifications, can provide a deeper understanding of the long-term effects of insecticide exposure on *D. gazella.* Investigating epigenetic changes can help uncover potential transgenerational impacts and provide insights into the persistence of altered behaviours.
6. The present study serves as a valuable foundation for future researchers interested in understanding alterations in dung beetle behaviour when exposed to various insecticides. Recent advancements in molecular mechanistics have paved the way for more comprehensive investigations.
7. The promise of continued development in this field suggests that future studies can build upon the current findings and address broader questions related to insecticide-induced behavioural changes in *D. gazella*, as well as potentially other insect species.

In summary, the future prospects for research on *D. gazella* offer a multifaceted approach, combining molecular, genetic, structural, and epigenetic studies. These avenues of exploration will contribute to a more comprehensive understanding of the effects of Deltamethrin and other insecticides on dung beetles, as well as potentially inform broader ecological and agricultural practices.