INTRODUCTION

Insects, which make up more than 75 percent of all documented animal species, may be found all over the globe and display a vast range of diversity in terms of both appearance and ecology (Balakrishnan, 2016). On a global scale, there exists an estimated 5.5 million species of insects, with around 1.5 million of these species belonging to the order Coleoptera (Stork, 2018). Scarabaeidae (Dung beetles) is one of the largest families in the order Coleoptera, with over 30,000 known species (Banerjee, 2014; Cajaiba et al., 2017) and three subfamilies totaling 6,850 known species (Chandra and Gupta, 2012, 2013; Stone et al., 2021). According to Piero and Dudenhoeffer, (2018), ancient Egyptians viewed scarab species as divine symbols of rebirth. In accordance with the spiritual beliefs of ancient Egyptians, it was believed that sunrays emanated from the head of the scarab beetle, while its dung ball symbolized the entirety of the earth. This concept represented a perpetual cycle of daily rejuvenation, forming the basis for a highly sustainable society. The taxonomic and ecological familiarity of dung beetles, as well as their sensitivity to changes in habitat management, makes them a useful ecological indicator group for biodiversity assessment (Nichols et al., 2007; Numa et al., 2009). We probably conceive of a dung beetle as an animal that carries a big ball of dung over the terrain. It is unlikely that we envision the fact that these widely distributed beetles are actively engaged in the process of consuming cow dung. However, empirical research suggests that this is really the case. Now scientists call them “nature’s pooper scooper” as they are helping farmers around the world to eliminate problems naturally and organically (Sanchez, 2019; Saha et al., 2021).

Dung beetles contributes a significant role to the nutrient cycle in pastures by playing a role in the removal and burial of dung from the surface to the soil, which serves as a source of nutrition for their offspring (Khadakkar et al., 2019). Dung beetles are classified into five main functional groups: paracoprids (or tunnellers) dig tunnels under the dung pat where they relocate dung masses; telecoprids (or rollers) build a brood ball, roll it away from the dung pat, and bury it. e.g. *Gymnopleurus melanarius*, *Sisyphus longipes*. Rollers are competitively superior to tunnellers, and tunnellers are competitively superior to dwellers (Pokhrel et al., 2021).; endocoprids (dwellers) nest and feed inside the dung pat or right beneath it in the dung–soil interface; lay eggs directly into the dung where the larvae spend the entirety of their development e.g. *Oniticellus cinctus*, *Drepanocerus setosus*, *Liatongus indicus* (Vinod and Sabu, 2007).non-nesters (also dwellers) feed on dung but do not make nests or nest elsewhere (e.g. on roots); and kleptocoprids (i.e. brood parasites) nest in the brood balls created by other species (González-Megías and SánchezPiñero, 2003; Fountain-Jones et al., 2015; Tonelli, 2021). The burrower exhibits very distinctive navigation behaviour and depends only on route integration as a means to determine the location of its burrow. Upon encountering a dung pile, the organisms in question exhibit a behaviour wherein they depart from the immediate vicinity and proceed to excavate a tunnel at a certain distance from the original location. To sustain their burrows, they engage in a series of trips to the dung source, accumulating material to form a pile. This behaviour has been seen in *Scarabaeus galenus*, as documented by Dacke et al., (2020) and Arellano et al., (2023).

The most unique nesting behaviour among dung beetles is tunneling, which refers to those species that burrow beneath the dung, either packing the tunnel with dung masses (each separated by a soil barrier) or excavating a chamber that houses one to several dung balls. These caches of dung contain the developing young, providing them with food and shelter. Tunneling species are the most beneficial to pasture health, enhancing the soil by increasing percolation, introducing organic matter into the soil, and nutrient cycling (Simmons and Ridsdill-Smith 2011; Gomez-Cifuentes et al., 2019). Tunneller beetles dig tunnels in the soil where they form small balls of dung known as brood balls. The female lays eggs into those brood balls, where the larvae will feed e.g. *Onthophagus bonansus*, *Onitis subopacus* (Gebert et al., 2019; Tonelli, 2021). There exists a competitive hierarchy among rollers, tunnellers, and dwellers, with rollers demonstrating greater competitive abilities over tunnellers, and tunnellers exhibiting superior competitive abilities over dwellers. In contrast, manure is relocated to greater depths within the soil profile by tunnellers (Anduaga and Huerta, 2007). In contrast, the behaviour of rollers and dwellers mostly involves dispersing the pad or, in the case of rollers, burying it within shallow tunnels (Doube and Marshall, 2014). According to Davis and Scholtz, (2001), the worldwide population of rollers and tunnellers, specifically belonging to the Coleoptera family’s Geotrupidae and Scarabaeidae, is estimated to be around 6,000 species. Additionally, Cabrero-Sañudo and Lobo, (2009) have identified a minimum of 2,000 species of dwellers, namely within the subfamily Aphodiinae. According to Pokhrel et al., (2021), the Afrotropical region exhibits the greatest abundance of authentic dung beetle species among the various biogeographical regions, comprising approximately 44% of the total. Subsequently, the Neotropical region accounts for 24%, the Oriental region for 14%, Australia for 9%, the Palearctic region for 7%, and the Nearctic region for 2%.

They feed on microorganism-rich mammalian feces to provide fibrous material for their larvae to nest. According to Holter, (2016), Frank et al., (2017), and Pandya et al., (2023), dung beetles exhibit a preference for omnivorous dung over herbivorous dung, with carnivore dung being the least favoured. The consumption and relocation of dung have been observed to exhibit diverse patterns, which are influenced by factors such as soil type and moisture (Nichols et al., 2008), dung quality (Braga et al., 2013), and pair cooperation (Slade et al., 2011). This phenomenon has been documented in various studies (De Groot et al., 2002; Banerjee, 2014; Tarasov and Dimitrov, 2016; Singh et al., 2019). Moreover, it has been shown that these organisms have the potential to augment soil fertility, improve soil permeability, promote plant development, facilitate seed dispersal, regulate parasite growth, and mitigate greenhouse gas emissions through the utilization of dung for sustenance and reproduction (Latha and Sabu, 2018). The decline in dung beetle diversity in forests and pastures as a result of human activities has been documented in several studies (Nichols et al., 2009; Basto-Estrella et al., 2014; Kim et al., 2021). This decline poses a threat to both the population of dung beetles and the ecosystem services they offer (Nichols et al., 2008). Despite the numerous ecological benefits associated with dung beetles, their populations are at risk due to anthropogenic activities. Therefore, it is crucial to do comprehensive research on dung beetles, since they play a vital role in preserving a well-functioning environment (Salomão et al., 2020).

Recent literature has mentioned about the programs that introduce dung beetles to an area improve the ecosystem services delivered by the beetles and make awareness that encourage the transfer of knowledge. Dung beetles reduce the potential for livestock and human parasites to develop in cow pens by dispersing, shredding, and burying dung Ryan et al., 2011). Certain pests have the ability to actively target animals in order to extract blood, while others serve as carriers of parasites, germs, and viruses that can cause disease in both humans and livestock. Multiple research done in controlled indoor or semi-natural field settings have shown evidence supporting the aforementioned advantages (Bishop et al., 2005; Gregory et al., 2015; Forgie et al., 2018;

Braga et al., 2013; Noriega et al., 2021; Barragán et al., 2022). Nevertheless, the task of establishing these advantages in real-world settings, where conditions are less controllable, presents a greater challenge.

Irrespective of their impact on pest species populations, dung beetles have an increased propensity to remove dung pads from the pasture surface when their population density is high (Giménez Gómez et al., 2018; Pecenka and Lundgren, 2018). According to Nichols et al., (2008), the act of burying dung by beetles contributes to the restoration of labile nutrients, such as nitrogen, phosphorus, and potassium, to plants. This process also enhances nutrient levels in the soil, improves soil aeration and water percolation, and mitigates the runoff of these nutrients into surface waters. The contribution of these services helped by introduced dung beetles is particularly significant in instances when native species do not completely exploit seasonal, local, or geographic niches (Ridsdill-Smith and Edwards, 2011). Dung beetles play a significant role in nutrient cycle by sequestering carbon, hence reducing carbon content and methane emissions in fresh dung pads (Iwasa et al., 2015).

Because of their distinct nesting habit, paracoprids are the most studied of all dung beetles. The species under consideration are mostly distributed in forested areas and agricultural environments (Sabu et al., 2006; 2007; Venugopal et al., 2012) worldwide (Andresen, 2005). *Digitonthophagus gazella* (*D. gazella*) (Fabricius, 1787) has been identified as the most proficient species in the removal of cow manure (Young, 2007). Additionally, it has the greatest reproductive rate among dung beetle species belonging to the Scarabaeinae family, characterized by a short generation cycle (Prameela and Sabu Thomas, 2020). Moreover, *D. gazella* exhibits competitive behaviours, possesses a notable dispersal capability (Ortega-Echeverría and Noriega, 2022), and demonstrates a broad tolerance to various climatic conditions. These attributes not only render it a suitable species for introduction in areas lacking its presence, but also contribute to its efficacy as a formidable competitor and invader (Filho et al., 2019). This particular kind of coprophagous beetle has a distinctive nesting behaviour. Biparental care is a prevalent and distinctive nesting behaviour observed across numerous dung beetle species. In this behaviour, both male and female individuals contribute to the care of larvae inside brood masses. Females, in particular, assume a significant role in providing care and protection to offspring, hence enhancing their survival by mitigating potential dangers. During reproduction, both members of

Onthophagus remove portions of excrement from the pad and compress it into the blind end of tunnels built beneath the pad. Subsequently, a solitary egg is placed into an enclosure specifically designed for egg storage, and subsequently shut. Each individual egg, along with its corresponding supply of excrement, becomes a collective unit known as a brood mass. A distinct sexual division of labor is observed in the allocation of time spent on parental care activities throughout the provisioning of a brood mass. Females dedicate around 84% of their time budget to these responsibilities, whereas males commit just 48% of their time (Hunts and Simmons, 2002; House et al., 2011).

The distribution of their presence is evident in numerous countries, such as Africa, America, Australia (Noriega et al., 2020), Arabia, Madagascar, Pakistan, and Sri Lanka (Chandra and Gupta, 2013). Additionally, there have been documented sightings in various regions of India (Sabu et al., 2011; Chandra et al., 2012; Pawara et al., 2012; Gupta et al., 2014; Thakkar and Parikh, 2016), including the Vadodara district in Gujarat (Singhal et al., 2018). Adult *D. gazella* exhibit a range of colors from yellow to mottled yellowish brown, displaying a distinct sexual dimorphism. Specifically, males possess slightly curved and acute horns, whereas females possess a prominently elevated ridge that extends between their eyes (Chandra and Gupta, 2013). Additionally, both males and females possess three pairs of homologous legs. Notably, the fore tibia of these individuals is characterized by a tooth-like structure, enabling them to effectively burrow and construct tunnels beneath dung and soil. These dung beetles create tunnels by digging, supplying dung to young in the form of brood balls at the blind end of each tunnel (Pulido and Zunino, 2007; Moczek, 2009; Khadakkar et al., 2019). Only one egg is laid in an egg chamber that is then sealed (Hunt and Simmons, 2000). The larval stage of development is characterized by the inhabitation in an enclosed chamber until the process of pupation occurs. The utilization of nutrient-rich and short-lived excrement by developing offspring facilitates distinct behavioural and physiological adjustments that contribute to sub-sociality and biparental behaviour (Arce et al., 2012; Panaitof et al., 2016; Heurta et al., 2013). This process offers protection to the offspring against competition and dehydration (Rauter and Moore, 2002; Kim et al., 2021).

The investigation of insect navigation and its underlying neurological mechanisms has been the subject of recent scholarly research (el Jundi et al., 2014; Webb and Wystrach, 2016).

These findings offer valuable perspectives on a basic inquiry within the field of neuroscience: the mechanisms by which the brain governs behaviour. Insects possess a strong orienting behaviour and exhibit physiological accessibility of their brains, making them very suitable creatures for future investigation of this inquiry. One notable illustration of such insects is the South African ball-rolling dung beetles, which rely on celestial cues as their primary visual reference (el Jundi et al., 2014b, 2015a; b). These beetles sustain their intended direction of travel by engaging in a dance (Baird et al., 2012), during which they capture an image of the sky and employ it as a blueprint for their navigational compass while rolling (el Jundi et al., 2016). The hierarchy of the primary celestial signals, namely polarized light and celestial body, has been determined in a group of neurons inside the central brain of dung beetles by a combination of behavioural tests and single neuron electrophysiological (el Jundi et al., 2015b). The area in the dung beetle brain where these neurons responsible for orientation, sometimes referred to as compass neurons, are located is called the central complex. This region is engaged in a diverse range of behaviours, as documented by several studies (Seelig and Jayaraman, 2015; Varga and Ritzmann, 2016). Similar to several other insect species, dung beetles likewise heavily depend on olfactory cues to locate their preferred food source, namely dung, as well as potential mates for reproduction (Tride and Burger, 2011; Burger, 2014).

According to Price and May (2009), their research findings indicate that the nesting behaviour of Phanaeus dung beetles can be attributed to the complex form of collaboration between males and females, which enhances the likelihood of successful nest construction. It has been suggested by scholars that foraging, mating, and nidificaution behaviour are contingent upon adaptive features that exert an impact on reproductive success. Moreover, in the case of adult dung rollers, they consume the same food that they supply to their larvae. This shared resource between male and female adults results in an inseparable connection between eating and reproductive behaviours. Nidification, within the context of Scarabaeinae, is a pivotal stage in the reproductive behaviour of this taxonomic group. The subfamily Scarabaeinae, namely the genera Phanaeus, Bolbites, Oxysternon, some Dichotomius, and Heliocopri, have been classified as Pattern II nesters. These insects construct nests that consist of one or more brood balls, each of which is created independently and housed within a separate chamber. The larvae undergo development within the brood ball, which is fortified by larval excrement and functions as the location for pupation. The establishment of the initial nesting gallery aligns with the development of the primary oocyte.

Despite their small brain size, insects exhibit remarkably intricate behaviour. Honey bees possess a repertoire of at least 59 discernible behavioural acts, surpassing the known range of any rodent species and constituting almost half of the behavioural repertoire observed in human children or bottlenose dolphins (Chittka and Niven, 2009). Insects possess the cognitive ability to acquire and apply rules, demonstrating the capability to generalize these principles across different sensory modalities. According to Schoofs et al., (2017), the sensory systems of these organisms frequently exhibit comparable or superior capabilities to our own. Dung beetles have been commonly employed as research subjects in investigations pertaining to phenotypic plasticity (Casasa and Moczek 2018; Stanbrook et al., 2021), male dimorphism (Laini et al., 2022), and the delivery of ecosystem services (Nichols et al., 2009; Smith et al., 2015).

Their behaviour has been studied almost exclusively within the framework of reproduction activities, such as the nest-staying behaviour of males (Akamine et al., 2016 and 2019), male–male combat , the tactics adopted by smaller males to avoid fights with larger ones and parental care displayed by females towards their brood balls (Macagno et al., 2018; Kim et al., 2021). Insect gene family expansion and contraction has been the research focus for several well-defined physiological genes and traits that are implicated in immunity, chemoreception, vision, feeding and digestion, detoxification, parasitism and reproduction (Holt et al., 2002; Wurm et al., 2011; Chen et al., 2016; McKenna et al., 2016; Papanicolaou et al. 2016; Martinson et al., 2017). With the exception of well-studied fly models such as *D. melanogaster* (Wigby et al., 2020) or *A. aegypti* (Ethan et al., 2019), however, relatively little is known about the genetic and functional aspects of neuroendocrine regulation and the molecular mechanism of the nesting behaviour in dung beetles. And to our knowledge there has been no detailed investigation for *D. gazella*.

Insect hormones contribute to caste differentiation via nutrition-sensing signal cascades during the larval stage and maintain the physiological state of the central nervous system and peripheral organs of the adults (Corona et al., 2016; Sasaki and Watanabe, 2022). These hormones are considered to act broadly on receptors in the brain and influence neuroendocrine systems that maintain sex-specific behaviours. To survive in a constantly changing environment, animals must adjust their behaviour depending on the external circumstances as well as their internal state. Appraisal of these cues triggers physiological, behavioural and cognitive phenomena that coordinate the individual's responses towards beneficial, but away from detrimental situations. Crucial for this goal is responding selectively to important stimuli, give attention to cues that matter and ignore others (Paulk et al., 2014).

Biogenic amines are derived from aromatic amino acids and function as neurotransmitters and neuromodulators in the central nervous system or can be released in circulation to act as (neuro) hormones, controlling behaviours and reproduction in insects. The phylogenetic distribution of these neuromodulators suggests a deep evolutionary history predating the origin of the nervous system (Gallo et al., 2016). These substances are synthesized in neurosecretory cells in the brain or other ganglia and secreted into the relevant neural circuit and other target tissues. In target cells, a specific amine binds with its receptors, changing the intracellular levels of secondary messengers, including cAMP, and resulting in the expression of relevant genes or changes in threshold for neuronal activation (Sakaki et al., 2021). The ubiquitous biogenic amines dopamine (DA), serotonin (5-HT), and octopamine (OA), activate neural circuitry to regulate behaviour (Libersat and Pflueger, 2004; Bergan, 2015; Kamhi et al., 2017).

In insects, monoamines are functionally varied with just minor structural alterations (Mustard et al., 2005; Blenau and Thamm, 2011). The regulation of behaviour in many insect orders is influenced by conserved aminergic circuits (Kravitz and Huber, 2003; Barron et al., 2010) and the expression patterns of receptors (Roeder, 1999; Blenau and Thamm, 2011). Nevertheless, there is a lack of understanding of how monoamine neurotransmitter systems have functioned as preadaptations in the evolutionary development of derived behaviours in dung beetles. According to Roshchina (2010), Cnidarians, Echinoderms, Molluscs, Worms, Arthropods, and Vertebrates all use dopamine (DA) as a neurotransmitter. Nevertheless, dopamine also serves crucial roles outside the confines of the neurological system. Dopamine (DA) has a pivotal role as a modulator of several behaviours and processes in insects. Dopamine is a significant neurotransmitter belonging to the catecholamine class, which is biochemically synthesized from tyrosine by the action of dopa decarboxylase. It is predominantly found in the central nervous system; however it may also be synthesized in epidermal cells. Dopamine exhibits conserved functions in regulating locomotion, pleasure, motivation, arousal, and memory across both invertebrate and vertebrate organisms. Melanisation and sclerotisation are essential biological processes that play a significant role in the development of the exoskeleton of insects, as well as in immunological function. Dopamine also assumes significant functions in social interactions, encompassing sexual relationships, across several insect species. Dopamine serves several roles in the regulation of social behaviour and facilitation of reproduction in eusocial Hymenoptera (Sasaki and Watanabe, 2022). Extensive research has been conducted on the Sex-Specific Regulatory Systems for Dopamine Production in the Honey Bee.

The process of 5-HT biosynthesis involves the enzymatic conversion of 5-hydroxytryptophan (5-HTP) to serotonin (5-HT) by the catalytic action of 5-HTP decarboxylase. The enzyme known as 5-HTP decarboxylase is functionally equivalent to dopamine decarboxylase, since it facilitates the decarboxylation process in the manufacture of dopamine. It is sometimes referred to as aromatic amino acid decarboxylase. The neurotransmitter 5-HT exerts significant influence on some facets of cognitive processes such as learning and memory, and plays a prominent role in regulating various behaviours shown by insects. The current body of research on the involvement of serotonin in insects remains quite restricted. However, existing studies have consistently emphasized the significant significance of serotonin signaling in a wide range of essential physiological processes in insects (Vleugels et al., 2014). Previous research has indicated that the inhibition of the serotonergic system in neurons of *D. melanogaster* has been linked to a decrease in performance of appetitive olfactory memory. Additionally, flies with genetically or pharmacologically reduced levels of 5-HT in the brain have exhibited a significant reduction in memory formation during a behavioural test. This test involved training the flies to avoid a chamber position that was associated with high temperature (Sitaraman et al., 2008 and 2012). The research conducted by Anstey et al., (2009) and Ott et al., (2012) shown that the presence of 5-HT and its downstream effector molecules in the desert locust species *Schistocerca gregaria* can trigger the manifestation of gregarious and swarming behaviour. 5-HT, or serotonin, is highly conserved signaling molecule throughout evolutionary history. Nevertheless, the mechanisms by which the regulatory systems of these amines govern nesting behaviour in dung beetles remain incompletely understood.

Neuropeptides can function as neurotransmitters, hormones, neuromodulators, and growth factors. Insects have a diverse array of neuropeptides. Certain members of this group have a notable similarity to neuropeptides found in vertebrates, including neuropeptide F, tachykinins, sulfakinins, and inotocin. Neural circuits can be modulated by the alteration of activity levels in constituent neurons or by modifying the synaptic effectiveness of the neural connections. The majority of examined insect neuropeptides have been found to be involved in many discrete behaviours, enabling them to process information from diverse stimuli and produce numerous behavioural responses. Neuropeptide Y (NPY) is known to exert regulatory control on several physiological processes, including as eating, reproductive functions, learning, and stress responses. Species-specific sensory signals and differences in the spatial and temporal expression of neuropeptides and their receptors can be used to account for the phenotypic variety in behavioural output. Moreover, a majority of neuropeptidergic cells exhibit the phenomenon of co-packaging neuropeptides within the same vesicles, resulting in their simultaneous release. Various behaviours require the coordinated action of several neuropeptides to effectively elicit the desired behavioural response. In order for the process of ecdysis to occur efficiently in *Manduca sexta,* a specific sequence of five neuropeptides (PETH, ETH, EH, CCAP, bursicon) is involved. Furthermore, it is worth noting that several neuropeptides, including kinins, corazonin, mips, npfs, and FMRFamides, may also have a significant impact in this context.

Based on the extensive participation of neurogenic amines and neuropeptides in diverse reproductive and social behaviours exhibited by insects (Roeder, 2005; Harano et al., 2008; Sasaki et al., 2012), our hypothesis posits that biogenic amines and neuropeptides are the most plausible contenders for facilitating the neuromodulatory regulation of parental behaviour in dung beetles. The utilization of abundant and temporary reproductive resources, such as dung, is believed to have facilitated the selection of distinct behavioural and physiological adaptations, ultimately resulting in the development of sub-sociality and biparental behaviour (Costa, 2018). The examination of parental care offers valuable insights into the intricate dynamics of caregiving, shedding light on the intricate interactions between ecological, social, and neuroendocrine elements in the control of behaviour. The extensive exploration of hormones in regulating reproductive social behaviour in vertebrates has been well-documented (Adkins-Regan, 2013). However, there is a lack of research on the potential influence of hormones on nesting behaviour in dung beetles, as highlighted by the recent study conducted by Pandya et al. (2022). This study demonstrated the involvement of neurotransmitters in modulating the nesting behaviour of the dung beetle species *D. gazella*. Despite this advancement, our understanding of the roles played by biogenic amines, neuropeptides, and the underlying molecular mechanisms remains limited. Therefore, the present study aims to investigate and elucidate the role of neurohormones and their mechanistic pathways in controlling nesting behaviour.

The impact of environmental disturbances on insect diversity and abundance has significant implications for the functioning of both natural and managed ecosystems (Winfree et al., 2015). One valuable conceptual framework for forecasting and comprehending the consequences of diminishing abundance and diversity on the functioning of ecosystems is the concept of effect traits. These are traits that exert an influence on a species' contribution to a particular ecosystem function (Lavorel and Garnier, 2002; Hallmann et al., 2017; Barmentlo et al., 2021). The utilization of pesticides in the agricultural sector is seeing a significant surge in emerging nations, particularly within Southeast Asia (Schreinemachers and Tipraqsa, 2012). India is a prominent producer of pesticides in the Asian region, with an annual production of 90,000 tonnes. It holds the twelfth place globally in terms of pesticide manufacture (Sharma et al., 2019).

Across the globe, the communities of coprophagous beetles are undergoing transformations due to the influence of human-induced stress. Extensive monitoring efforts have revealed a prevailing trend of population loss among these beetles (Sánchez-Bayo and Wyckhuys, 2019; Cavallaro et al., 2023). Dung beetles are recognized as significant bio-indicator of ecosystem health due to their many ecological activities, which exhibit taxonomic variations. Dung is a transient resource, and its spatial dependability varies across different scales, ranging from local to landscape levels. When contemplating the potential for repeated instances of pesticide exposure and taking into account the life history strategies of dung beetles, it becomes evident that airborne active ingredients, which are transported through seed dust or contaminated particulate matter originating from livestock feedlots and surface soils that have been directly planted, exposed to nearby agricultural runoff, or subjected to dusting, present a significant risk to the survival of these beetles. Moreover, it is important to note that dung beetles and the ecological processes they contribute to are susceptible to veterinary parasiticides inside agroecosystems.

When parasiticides, such as macrocyclic lactones and synthetic pyrethroids, are administered to livestock, they are excreted in dung with significant metabolic changes (Lumaret et al., 2012). The exposure to these compounds can result in an increased permeability of nerve and muscle cells to chloride ions, leading to hyperpolarization and a subsequent limitation of action potentials (Shoop and Soll, 2002). Consequently, this can cause a range of negative effects on dung beetles, including loss of olfaction, reduced fecundity, impaired movement, and ultimately death (Verdú et al., 2015; Martínez et al., 2017). In the context of veterinary medicine and the management of parasites, a common practice among farmers involves the utilization of pour-on solutions to combat flies and parasites. These applications frequently consist of macrocyclic lactones (such as ivermectin and eprinomectin) or pyrethroids (including deltamethrin and cypermethrin). The migration of macrocyclic lactones through the skin and subsequent excretion in dung has been documented in studies conducted by Tixier et al., (2016) and Virlouvet et al., (2006). This process has been found to have a significant detrimental impact on the populations of flies and beetles, as reported by Vale et al., (2004) and Virlouvet et al., (2006). The presence of beetles has been shown to have adverse consequences, as evidenced by studies conducted by Buijs et al., (2022) and Bruinenberg et al., (2023), even when the levels are below the limits of detection.

Nevertheless, recent studies have indicated the presence of insecticides and/or other pesticides in animal manure (Wohde et al., 2016; Buijs and Samwel-Mantingh, 2019). This finding raises concerns about the potential impact on insects that inhabit and feed on dung. The origins of the pesticides found in feces are frequently ambiguous. The primary sources that are most probable are (i) the application of veterinary medication and anti-parasitic medicines to combat ticks, flies, or helminths, and (ii) the inclusion of pesticides in animal feed. The extensive utilization of medicinal treatments targeting parasites may therefore result in elevated concentrations of these substances in dung, reaching levels that are detrimental to the survival of dung fauna and/or impede the reproductive capabilities of insects. Regarding the presence of pesticides in animal excrement, it has been noted that some types of animal feed, including concentrates, grains, and by-products, may include traces of pesticides. In a study conducted by

Walorczyk, (2008), a total of 15 chemical compounds were detected in 145 samples of animal feed. Furthermore, in a subsequent study by Walorczyk and Drożdżyński, (2012), it was shown that residues of insecticides were present in 17% of the 900 feed samples analyzed. The presence of insecticides in animal feed, which are subsequently ingested and expelled in dung or urine, may have adverse implications for the suitability of dung as a feeding and breeding environment for insects.

Insecticides are chemical compounds specifically formulated to selectively eliminate insects, mostly by interfering with essential physiological mechanisms within their bodies. Several scholarly investigations have shown the adverse impacts of insecticides, namely pyrethroids, on the population sizes of larvae and adult beetles, as evidenced by research conducted by Casida et al., (2013), Mann et al., (2015), Vale et al., (2015), Sands et al., (2018), Bruinenberg et al., (2023). A portion of this decrease in population can be ascribed to an escalation in agricultural practices and the fragmentation of natural habitats (Filgueiras et al., 2011). Nevertheless, the drop in cow populations may have been influenced by the presence of chemicals utilized for pest and parasite control. These compounds, when excreted in dung, can reach hazardous levels and can harm non-target organisms through both fatal and sub-lethal effects (Jacobs and Scholtz, 2015). Livestock are often treated with insecticides to manage pests and parasites. These insecticides can be eliminated from the body through feces and may provide a lethal threat to coprophagous insects, especially dung beetles, which play a significant ecological role. While there has been extensive research conducted on the effects of macrocyclic lactones, there has been comparatively less emphasis on investigating the potential impacts of pyrethroid insecticides. Pyrethroids are a prominent class of chemicals commonly employed in livestock husbandry to manage nuisance flies and ectoparasites (Pickett, 2004). In order to mitigate the detrimental impacts of pesticide exposure, insects adopt several compensatory behavioural and physiological strategies.

Insects, as a whole, are confronted with several stressors, such as chemicals, heat, and nutrition shortage, which are frequently encountered at suboptimal levels. The phenomenon of mild stress exposure inducing hormetic effects in insects has gained significant recognition in academic circles. This recognition has led to a deeper understanding of the consequences of such effects for insect management, as well as the broader ecological structure and function. The elucidation of the molecular foundations of hormesis in insects is currently gaining traction, providing insights into the many phenotypic hormetic reactions observed in insect reproduction, development, and behaviour (Cuttler et al., 2022). Multiple research investigations have demonstrated that a variety of chemical substances, including synthetic pesticides, have the potential to enhance the antioxidant activity in insects when they are subjected to moderate doses. This finding aligns with the idea of hormesis (Després et al., 2007; Gui et al., 2009; James and Xu, 2012).

The induction of oxidative stress is a recognized mechanism via which pesticides can have harmful effects on insects. This process involves the formation of reactive oxygen species (ROS), which subsequently disrupts the electrochemical gradient at the cellular membrane (Chen et al., 2010; Faize et al., 2011; Fatma et al., 2018). Krůček et al. (2015) have identified many pesticide classes, such as pyrethroids, organophosphates, and neonicotinoids that have the potential to stimulate the generation of reactive oxygen species (ROS). Reactive oxygen species (ROS) encompass a range of molecules, including superoxide radicals (O2-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH-). These molecules exhibit a high reactivity and have the potential to cause damage to different cellular constituents, such as DNA, proteins, and lipids. The occurrence of oxidative damage has the potential to interfere with critical cellular processes diminish reproductive efficacy, and induce cellular demise or impairment (Dickinson and Chang, 2011; Blount et al., 2016). Insects possess an inherent capacity in the form of an antioxidative response system to protect themselves from damage induced by reactive oxygen species (ROS). The antioxidant response system plays a crucial role in the detoxification of reactive oxygen species (ROS). It consists of both low-molecular antioxidants and antioxidant enzymes, which effectively delay and prevent the damaging effects of oxidative agents on biomolecules (Halliwell and Gutteridge, 2015). The participation of these entities in cell-signaling pathways that regulate programmed cell death, gene expression, and immunological defense systems is crucial for the maintenance of homeostasis (Gupta et al., 2010; Świętek et al., 2019). According to Lai et al., (2012), there is a direct relationship between the quantity of reactive oxygen species (ROS) present in a cell and the enzymatic activity. This suggests that the antioxidative pathway plays a crucial role in counteracting oxidative stress. Moreover, the generation and subsequent augmentation of reactive oxygen species (ROS) can transpire via various mechanisms. These mechanisms encompass the inhibition of antioxidant enzymes, such as superoxide dismutase (SOD) or glutathione peroxidase (GPx), as well as the direct promotion of ROS production through interactions with cellular components, including mitochondria or the electron transport chain (Yildiztekin et al., 2015). Superoxide dismutase (SOD) is an enzyme that plays a crucial role in the first defensive mechanism against reactive oxygen species (ROS). Its primary function is to facilitate the conversion of the superoxide radical (O_{2-}) into hydrogen peroxide (H_2O_2) and oxygen (O_2). This enzymatic activity effectively hinders the generation of highly reactive hydroxyl radicals, which are known to be extremely reactive and potentially damaging to cellular components. Additionally, the Catalase (CAT) enzyme facilitates the chemical reaction in which hydrogen peroxide (H_2O_2) is broken down into water (H_2O) and oxygen (O_2). This enzymatic process serves to avoid the build-up of H_2O_2, which has the potential to generate hydroxyl radicals. Glutathione-S-transferases (GSTs) demonstrate peroxidase activity and play a significant role in detoxification processes, hence contributing to the defense against reactive oxygen species (ROS). The presence of heightened oxidoreductase enzyme activity indicates the breakdown of pesticides, so indicating both the level of toxicity and the capacity to withstand the associated stress. Furthermore, Lipid peroxidase (LPO) is an enzymatic antioxidant that plays a crucial role in the neutralization of reactive oxygen species (ROS). The potential involvement of LPO in the process of detoxifying hydrogen peroxide (H2O2) during oxidative stress generated by pesticides is indicated by a significant rise in its activity, which has been extensively investigated in previous studies.

Cytochrome P450 (CYP450) enzymes are a diverse group of enzymes that are ubiquitously present in a wide range of animals, encompassing insects among other taxa. Within the realm of insects, many families of CYP450 enzymes have been recognized to have significant importance since they are involved in the metabolic processes of both endogenous substances and xenobiotics, encompassing commercially utilized pesticides (Mao et al., 2009). Moreover, continuing research is being conducted on the discovery and characterization of CYP450 enzymes in insects, specifically beetles. Detoxification enzymes, specifically Cytochrome P450 monooxygenases (CYP450) and glutathione S-transferases , are of significant importance in the metabolism of insecticides. These enzymes are actively expressed in scarab beetle taxa that are considered pests. Notably, the induction of CYP450 serves as a crucial mechanism for the detoxification of neonicotinoids, as highlighted in studies conducted by Cassida, (2011), Adesanya et al., (2018), and Cavallaro et al., (2023). It is worth mentioning that the stimulation of CYP450 enzymes plays a crucial role in the process of pesticide detoxification.

Oxidative stress, recognized as a primary contributor to cognitive decline, has been extensively studied in this context (Fukui et al., 2002; Praticò, 2008). The field of ecotoxicology has made significant advancements, yielding a diverse array of biomarkers for assessing pesticide exposure and its associated impacts. One such biomarker is the use of organosomatic indices, which provide valuable insights into individual fitness (Norrdahl et al., 2004). Organosomatic indices, calculated by comparing organ weight to body weight, are particularly relevant for evaluating the adverse effects of chemicals on target organs (Giullo and Hinton, 2008). These indices offer indicators of the physiological state of specimens, reflecting factors such as fat accumulation, gonad development, overall health, and responses to pesticide-induced toxicities (Upadhyay et al., 2018). Analyzing changes in organ weight relative to body mass and correlating these findings with tissue histomorphology is instrumental in comprehending the toxic effects of Deltamethrin. Notably, prolonged exposure periods to pesticides may have a consequence on its body tissue weight (Bhartia and Rasool, 2021). So, in addition to organosomatic indices, cytological and histopathological alterations in non-target insects exposed to insecticides have been well-documented. These alterations include changes in the midgut of species like Apis mellifera (Catae et al., 2014), Calliabaetis radiates (Gutierrez et al., 2016), Ceraeochrysa claveri (Scudeler and dos Santos, 2013), and Chironomus calligraphus (Lavarías et al., 2017) following exposure to various pesticides.

Furthermore, insecticides have been found in soil and dung in pasturelands, raising concerns about their unintended exposure to dung beetles as non-target organisms (Mann et al., 2015). This mode of exposure is particularly concerning as it may impact the central nervous system and brain development due to the brain's vulnerability to oxidative damage caused by free radicals (Anderson, 2004). Insecticide exposure has also been linked to alterations in chemical communication systems, affecting various insect behaviours such as foraging, oviposition site selection, and pheromone communication. These changes are driven by intricate physiological mechanisms involving hormones and neurohormones, ultimately reducing insect reproduction rates (Price et al., 2011; Candolin and Wong, 2012; Benelli et al., 2014; Schoonhoven, 2018).

Pyrethroids can alter neurotransmitter levels and metabolites in the insect brain (Kori et al., 2018). Neuropeptides, on the other hand, regulate various physiological processes in insects, including responses to stress induced by insecticides (Li et al., 2020; Schoofs et al., 2017; Ragionieri et al., 2022). They are released as chemical signals during stressful conditions and govern behavioural responses. Neuropeptide-like peptides such as npf, it, and mip have been linked to stress responses and their regulation (Schoofs et al., 2017; Ragionieri et al., 2022). Studies using molecular genetics techniques have investigated the roles of neuropeptides and their receptors in various insect species, shedding light on their functions in behaviour and physiology (Nässel and Homberg, 2006; Ragionieri et al., 2022; Pandit et al., 2018; Ragionieri and Predel, 2020). Insecticide exposure can disrupt chemical communication systems in insects, leading to changes in behaviours like foraging, oviposition site selection, and pheromone communication. These changes are driven by complex physiological mechanisms involving hormones and neurohormones, ultimately affecting reproductive success (Wei et al., 2004). However, there are lacunae of information as far as the effect of Deltamethrin on *D. gazella* is concerned.

Thus, considering the points mentioned above, the present study was aimed to investigate the neural regulation in the nesting behaviour of dung beetle (D. gazella) on exposure to insecticide (Deltamethrin). To fulfill this aim, the objectives were as follows:

1) Brood morphometry and digging behaviour (Chapter 1)
2) Understanding the role of neurohormones in the nesting behaviour of dung beetle (*D. gazella*) (Chapter 2)
3) Toxic impacts of Deltamethrin on biochemical and histological alterations (Chapter 3)
4) Neurophysiological alterations in the nesting behaviour of dung beetle (*D. gazella*) on exposure to Deltamethrin (Chapter 4)