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ORIGINAL RESEARCH ARTICLE

Elucidating the role of neurotransmitters in the nesting behaviour of *Digitonthophagus gazella* (Fabricius, 1787) (Coleoptera: Scarabaeidae)

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Abstract

Dung beetles belonging to the subfamily Scarabaeinae are ecologically important organisms that feed primarily on mammalian dung for their nesting and brooding. The paracoprids are characterized for their complex tunnel-making behaviour. The present study revealed the role of neurotransmitters in the nesting behaviour of *Digitonthophagus gazella* (Fabricius in Mantissa insectorum sistens species nuper detectas adiectis synonymis, observation ibus, descriptionibus, emendationibus, 1787) (Coleoptera: Scarabaeidae) under laboratory conditions. The development period was observed to be 31 days, comprising of four stages- egg, larva (1st instar, 2nd instar, and 3rd instar), pupa, and adult. The nesting pattern of *D. gazella* showed a time dependent increase in the length (14.7 ± 0.1 cm, 16.9 ± 0.1 cm and 19.8 ± 0.1 cm), and total depth (9.8 ± 0.1 cm, 12.9 ± 0.1 cm and 13.5 ± 0.1 cm) of the tunnel on 10th, 20th, and 30th day. Estimation of the neurotransmitters revealed that acetylcholine esterase (AChE), biogenic amines- 5-hydroxytryptamine serotonin (5-HT); dopamine (DA), and nitric oxide (NO) increased significantly ($p < 0.01$) in a time dependent manner in both male and female, suggesting their role in parental behaviour. These results provide the first evidence for a potential role of neurotransmitters in the modulation of nesting behaviour of the dung beetle; *D. gazella*.

Keywords Dung beetles · Brood morphometry · Nesting behaviour · Tunnel pattern · Acetylcholine esterase · Nitric Oxide · Dopamine · Serotonin

Introduction

The family Scarabaeidae (Dung beetles) is one of the largest families of order Coleoptera, which contains more than 30,000 species in the world (Banerjee 2014; Cajaiba et al. 2017). It comprises of nearly 27,800 species worldwide (Chandra and Gupta 2012a, b, c, d, 2013) with two subfamilies Aphodiinae and Scarabaeinae including approximately 6,850 species worldwide (Ratcliffe and Jameson 2001; Chandra and Gupta 2013). Earlier, the taxonomic studies on Indian Scarabaeinae were carried out by Arrow (1931), Paulian (1945, 1980, 1983), Balthasar (1963, 1974), Mikšić (1977), Endrodi (1985), Kuijten (1983), Chandra (1986, 1999), and Krikken (2009), Sabu et al. (2011). Later, comprehensive work on the diversity of dung beetles was conducted by Chandra and Ahirwar (2007), Chandra and Singh (2010), and Chandra and Gupta (2011, 2012a, b, c) and have recorded 124 species belonging to 45 genera in 11 subfamilies from Madhya Pradesh and Chhattisgarh. Thakkar and Parikh (2016), and Singhal et al. (2018) have also recorded species of dung beetles from Gujarat.

Dung beetles exhibit a wide range of ecological functions (Kakkar and Gupta 2009; Brown et al. 2010; Gullan and Cranston 2010), morphological as well as behavioural adaptations which makes them universally distributed. They carry out dung decomposition by feeding on dung and by performing dung burial activity. Such burial activity and their pasture productivity (Hernández et al. 2011) have classified them into four types: Telecoprid (rollers),

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Endocoprid (burrowers), Kleptocoprids (dwellers), and Paracoprid (tunellers) (Doube 1990). They feed on micro-organism rich mammalian dung as a source of fibrous material to brood their larvae. Mostly dung beetles prefer omnivore, then herbivore dung and least preferred is carnivore dung (Frank et al. 2017). Depending upon soil type, and moisture (Nichols et al. 2008), dung quality (Braga et al. 2013), and pair cooperation (Slade et al. 2011), they use diverse pattern of consumption and relocation of dung (De Groot et al. 2002; Banerjee 2014; Tarasov and Dimitrov 2016; Singh et al. 2019). They are known to enhance soil fertility, soil permeability, plant growth, seed dispersal, control parasitic growth, and reducing the emission of greenhouse gases by utilizing the dung for food and reproduction (Latha and Sabu 2018). Despite of numerous ecological benefits it provides, a decline in dung beetle diversity is recently documented due to anthropogenic activities observed in forests and pastures (Martínez et al. 2001; Nichols et al. 2009; Basto-Estrella et al. 2014). These declines entail jeopardy for the population of dung beetles and the ecosystem services they provide (Nichols et al. 2008). Therefore, it becomes imperative to carry out more studies on dung beetles which are an essential bio-indicator (Salomão et al. 2020) in maintaining a healthy ecosystem by performing unique behaviour. Nevertheless, most studies have unravelled the diversity and distribution of these species, and the ecosystem services they provide, but there is a lacuna in the work done on the physiological parameters involved in regulating behaviour of individual dung beetles. Hence, in the present study, dung beetles were used to study the neuroendocrine mechanism involved in the behaviour pattern.

Of all the types of dung beetles, paracoprids are in attention due to their unique pattern of nesting. They are found predominantly in the forest and agri habitats (Sabu et al. 2006; 2007; Venugopal et al. 2012) across the globe (Andresen 2005). *Digitonthophagus gazella* (Fabricius 1787), a paracoprid shows a unique behaviour of nesting. Their presence is well marked in many countries including Africa, America, introduced in Australia (Noriega et al. 2020) Arabia, Madagascar, Pakistan and Sri Lanka (Chandra and Gupta 2013), however it has also been recorded in many parts of India (Sabu et al. 2011; Chandra et al. 2012; Pawara et al. 2012; Gupta et al. 2014; Thakkar and Parikh, 2016) including Vadodara district (Singhal et al. 2018), Gujarat, which makes them a suitable model for the study. Adult *D. gazella* are yellow to mottled yellowish brown in colour and show a complete sexual dimorphism. Males have slightely curved and acute horns while the female have a strongly elevated ridge that extends between the eyes (Chandra and Gupta 2013). These dung beetles excavate tunnels and provide dung to offspring in the form of brood balls at the blind end of each tunnel (Pulido-Herrera and Zunino 2007; Moczek 2009; Khadakkar et al. 2019), with only a single egg deposited into an egg chamber and sealed. Utilization of rich and ephemeral dung promotes unique behavioural and physiological adaptations leading to sub sociality and biparental behaviour (Arce et al. 2012; Panaitof et al. 2016).

The pervasive role of neurotransmitters in reproductive behaviour have been explored extensively in vertebrates (Adkins-Regan 2005), however, this aspects are meagerly investigated in insects (Riddiford 2012). Baring a few reports, neurotransmitters like Acetylcholine, Serotonin, Dopamine, and Nitric Oxide are reported in insects such as *Drosophila*, *Manduca sexta*, *Anopheles gambiae* , *Anopheles stephensi* (Müller 1996; Jacklet 1997; Charpentier et al. 2000; Davies 2000; Bicker 2001a, b; Vleugels et al. 2015). Biogenic amines, DA, and 5-HT has been extensively explored in the social context-dependent fighting behaviour, and territorial dominance in cricket, *Gryllus bimaculatus* (Dyakonova and Krushinsky 2013), fruit fly, *Drosophila melanogaster* (Alekseyenko et al. 2014; Zwarts et al. 2012), stalkeyed fly, *Teleopsis dalmanni* (Bubak et al. 2014a, b; Casasa et al. 2017). Further, DA, and 5-HT have been accounted to play a vital role in cuticle sclerotization, melanisation, and social interaction (Beggs and Mercer 2009; Andersen 2010; Vleugels et al. 2015; Verlinden 2018; Singhal et al. 2019). Given the robust links established between 5-HT and DA and the modulation of behavioural state, we hypothesize that the biogenic amines DA and 5-HT represents the most likely candidates for the neuromodulatory control of nesting behaviour in *D. gazella.*

Therefore, in the present study, we have experimentally evaluated the role of neurotransmitters in the nesting behaviour of *D. gazella*. We selected *D. gazella* because they showed unique pattern of tunneling, can be reared in laboratory and since no study has been conducted on these species with the present aspect.

Materials and methods

Collection, acclimatization, and rearing of *Digitonthophagus gazella*

Digitonthophagus gazella were collected from the agricultural fields of Channi (22.363˚N, 73.166˚E), Sindhrot (22.331˚N, 73.063˚E), and Timbi (23.149˚7 N, 74.002˚E) of Vadodara city, located in Western India (Fig. 1). Collection of *D. gazella* was carried out during the time of dawn and dusk, in the months of June to November for three years (2018–21). The dung beetles were collected by using the handpick method from the dung pats, and dung heaps, and by digging the soil under the dung pats with the help of shovel/trowel (30 cm), and were brought to laboratory for identification and rearing. Morphological identification was done upto the species level with the help of standard taxonomic keys (Arrow 1931; Balthasar 1963; Chandra and Gupta 2013) and by comparing with the specimens in Department Repository. Dung beetles (12–14 mm long, 7–8 mm wide) were maintained under laboratory conditions in earthen pots following the method proposed by Gaikwad and Bhawane (2015). Rearing medium in the pots was the sandy soil (obtained from collection sites, pH-6.8) and fresh dung of the cattle, used as food resource for the dung beetles. Fresh dung of buffalo [rich in carbohydrate content (Prevoius work in our lab-Unpublished)] was obtained from the stable with the help of trowel (30 cm), near the same agricultural fields of beetles' collection, and a 250 g of dung was added to the pot regularly. Further, these earthen pots were covered with a black cloth at the top (Fig. 2), and placed in a large plastic tray containing moist sand for maintaining the temperature (22° to 26 °C), and humidity (70%), with a 10L:12D light regime (Bang et al. 2004).

Experimental setup for tunnel pattern

After acclimatization and rearing, 10 dung beetles (5 males and 5 females of same size and weight) were released in the earthen pots and were monitored for the appearance of the holes on the dung layer. The burrow cast were excavated at the end of 10^{th}, 20^{th}, and 30^{th} day. The casting and measurement of the tunnel was done following the method of Sinha (2013). Measurements of the number of openings, length, total depth, diameter, number of branches, and total area of the burrow was determined by the following formula:

$$Area = \frac{\pi \times a \times b}{4}$$

where a is the length of the burrow opening, b is the width of burrow opening.

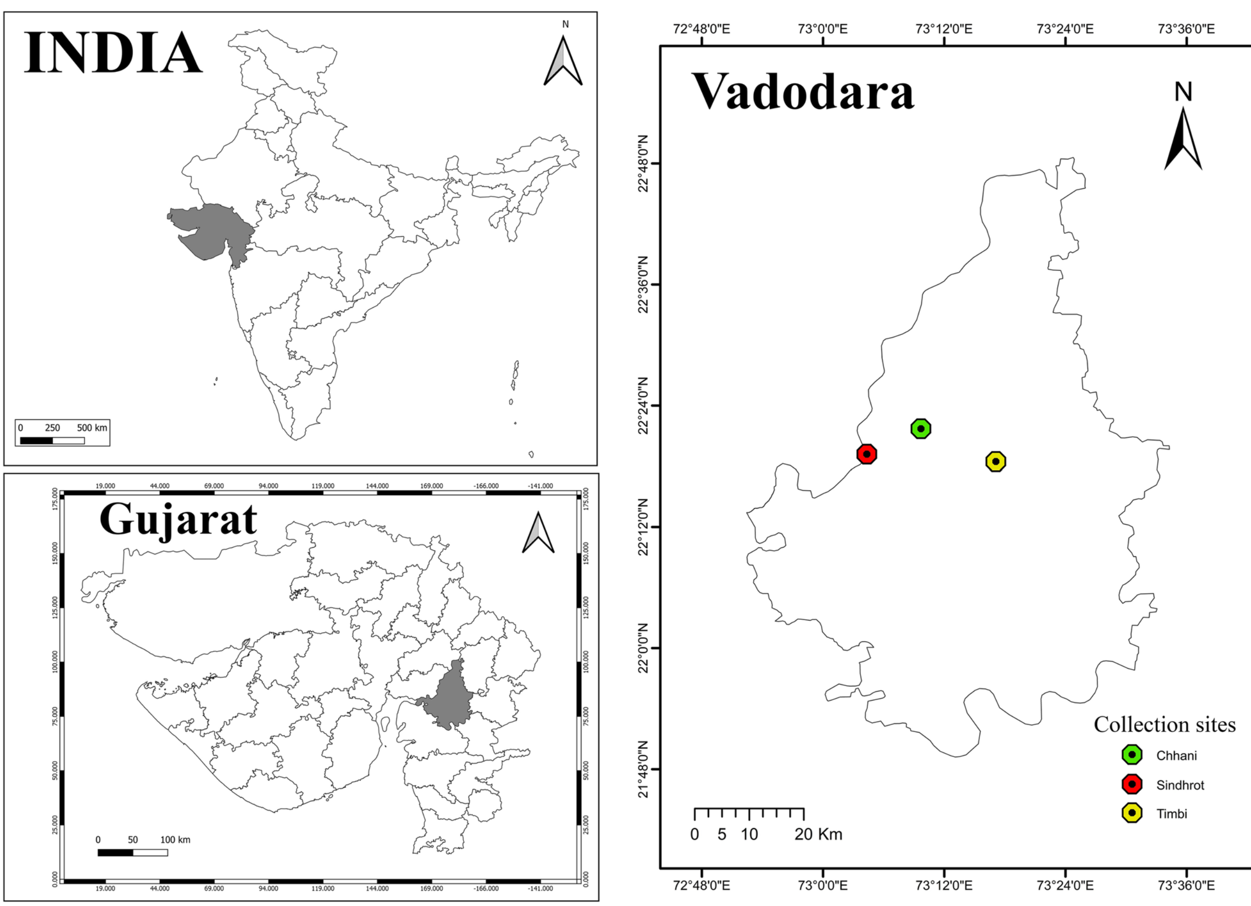


Fig. 1 Map represents the collection sites for *D. gazella* from Vadodara district of Gujarat, India. *D. gazella* were collected from the outskirts of Vadodara district such as Channi (22.363˚N, 73.166˚E), Sindhrot (22.331˚N, 73.063˚E), and Timbi (23.149˚7 N, 74.002˚E)

Fig. 2 Rearing medium for dung beetle. *D. gazella* were allowed to acclimatize in the convinient size earthen pot placed in the mud tray. Temperature (26 °C) and humidity (70%) was maintained within this medium

Life cycle study and brood morphometry

For life cycle study and brood morphometry, five pair of adults (14 mm long) were released in earthen pot. The tunnel pattern was observed on 10^{th}, 20^{th}, and 30^{th} day, and the broodballs formed were collected for the morphometry (length, diameter, and weight). At 12 h interval, broodballs were monitored for the development of the individuals, starting from egg up to the adult stage. The opening in the brood balls was immediately sealed after observation with the help of fresh dung. Thenafter, length, and weight measurements of each stage of development were noted with the help of vernier caliper (Zhart, India) and analytical balance (Wensar, PGB200, India) (Singh et al. 2019).

Estimation of neurotransmitters

After 10^{th}, 20^{th}, and 30^{th} day of tunneling, each pair (of 5 pairs) of male and female *D. gazella* were sacrificed, and the brain was dissected in ice-cold saline (pH-7.4). Further, the rate of AChE activity was measured according to the method described earlier by Ellman et al. (1961) and NO levels were estimated by following the method of Miranda et al. (2001). Biogenic amines (DA and 5-HT) estimation was carried out following the method of Schlumpf et al. (1974). Similar procedure was followed for the non-breeding beetles (control), where male and female beetles were kept in separate earthen pots.

Statistical analysis

The computed data was analyzed using PRISM 6 software. ANOVA was used to compare between groups followed by DUNNETS multiple comparison tests to test the significant differences among the individual treatment combinations. Statistical significance was accepted at $p \leq 0.05$ for the analysis.

Results

Nesting behaviour

During the period of acclimatization and rearing, the dung beetles spent most of the time feeding and constructing the nest. On the second day of their release, both male and female dung beetles started constructing the tunnel and carrying dung (brood balls) along the tunnel. Males were observed more frequently on the surface of dung and females were seen occasionally. Eventually, the appearance of holes over the dung layer was the confirmation of tunnel formation and egg-laying.

Tunnel pattern

Construction of the tunnel was carried out by both males and females underneath the dung. Observations of the tunnel obtained at three different time points (10^{th}, 20^{th}, and 30^{th} day) are given in Table 1. Under laboratory conditions, the tunnel pattern studies indicates that *D. gazella* constructs a simple tunnel over the period of time (Fig. 3A). The total depth, length, and area of the burrow cast were found to be significantly ($p < 0.05$) increasing with increasing period of time (Fig. 3B).

Brood morphometry

Tunnels were dug and the brood balls were removed. The average number (Mean ± SD) of the brood ball was found

Table 1 Observation of burrow cast of *D. gazella* on 10^{th}, 20^{th} and 30^{th} day

Sr. no	Observations	10 days	20 days	30 days
1	**NBO**	1	1	1
2	**L**	14.7	16.9	19.8
3	**TD**	9.8	12.9	13.5
4	**DOB**	1.11	1.11	1.16
5	**Area**	12.8	14.72	18.02
6	**NOB**	3	4	4
7	**Pattern**	Simple	Simple	Simple

NBO Number of Burrow Openings, *L* Length (cm), *TD* Total Depth (cm), *D* Diameter of burrow (cm), *Area* (cm^2), *NOB* Number of Branches of burrows

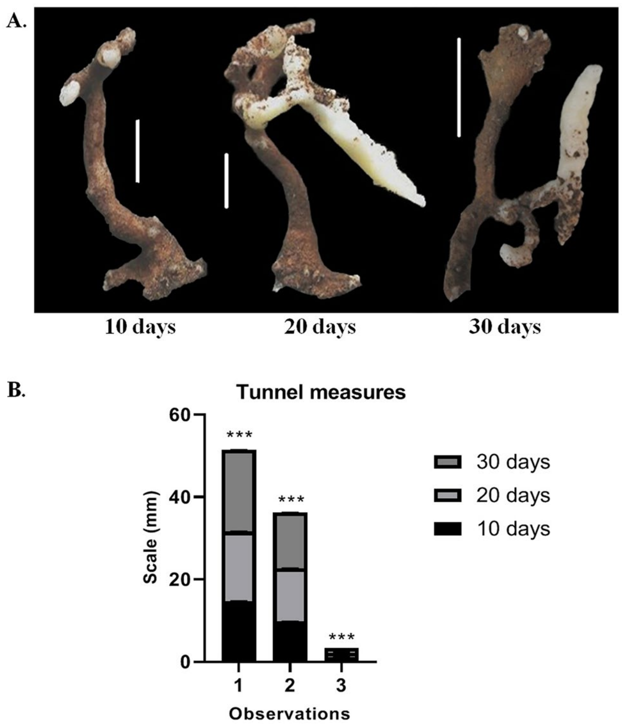


Fig. 3 The tunnel pattern of *D. gazella* is shown. **A** The tunnel formed at the end of the 10th, 20th, and 30th day is shown (scale = 2 cm). **B** The graph represents the key measures of tunnel formation. The length (1), depth (2), and diameter (3) of the tunnel were observed to increase significantly ($p < 0.001$) with the increasing number of days (10th, 20th, and 30.th day). *Here,* $p < 0.001$*** was obtained from statistical analysis done using two-way ANOVA *(n = 3)*

to be 10 ± 2 to 34 ± 0.69 from 7 to 30 days. Brood morphometry showed that the brood balls were spherical with strongly stacked dung containing a single egg at the centre of the ball (Fig. 4A). Cylindrical shaped brood masses (Mean ± SD; n = 15) had a length of 33.72 ± 5.89 mm, a width of 7.8 ± 0.89 mm, and weighed 745 ± 1.34 mg (Table 2).

Life cycle

The duration of different developmental stages are presented in Fig. 4B (Table I of the Supplementary data). The life cycle of *D. gazella* comprised of 4 stages, *i.e.*, egg, larva (1st instar, 2nd instar, and 3rd instar), pupa, and adult (Fig. 4C). The total development period was found to be of 31 days (Supplementary table I). Further, the length, diameter, and weight of all the developing stages were recorded (Table 2). Each elongated, cylindrical brood ball showed a single egg laid in the central chamber in a vertical position. The eggs were elongated, cylindrical, and creamy white. After 2–4 days, the larva was transparent, with only the tips of the mandibles being dark brown. The first instar larvae had its characteristic hump, which was used as a pivot when fed on the dung. The second instar larva showed characteristic mandibles. 3rd instar was observed to comprise of the highest length and weight where as the highest diameter was found to be of pupa (Fig. 4D). The newly developed pupa was creamy white and shiny, sexual dimorphism was evident in the pupa. The pupae of male had two horns on the head and a median projection, whereas the female had only a median projection. The pupal stage lasted for 25–27 days, followed by its transformation into adult. After the emergence from the brood mass, adults showed pigmentation and maturation within 2–3 days and its longevity period was 60–82 days. Complete sexual dimorphism was observed in adults where in males had vertical, elongated horns between the eyes and protibia was found to be slightly curved medially; females had a strong elevated ridge between eyes on the head with less slightly curved protibia.

Alterations in neurotransmitter levels during nesting behaviour

To have an insight into whether there is any significant role of the neurotransmitters in the nesting behaviour of *D. gazella*, brain levels of AChE, 5-HT, DA, and NO were biochemically analyzed. A significant time-dependent increase in all the neurotransmitters was observed on the 10th, 20th, and 30th day of introduction of *D. gazella* into the experimental setup as compared to control (Fig. 5). The lowest level of neurotransmitters was recorded for the control group and the maximum increase in the levels of neurotransmitters was found to be on the 30th day in male and female *D. gazella*. 5-HT was found to be higher in males compared to females, whereas the levels of DA were more in females compared to males.

Discussion

Digitonthophagus gazella is best known for its behaviour of removing the dung from the pat and compacting it in tunnels for provisioning to their offspring. The present study has proved that the nesting behaviour (tunnel formation, broodmass formation, and parental care) by *D. gazella* is similar to other *Onthophagus* species (Huerta and García-Hernández 2013; Arellano et al. 2017; Sane et al. 2020); however, there are few differences observed. In the present study, a time-dependent complexity in the formation of tunnel was observed. On 10th day, the tunnel consisted of only 3 branches which was found to increase on 20th and 30th day resulting into 4 branches, housing linearly arranged brood masses. A time-dependent increase in the length and total depth was also observed. Sane et al. (2020) in their studies of structural diversity and behavioural principles on insect architecture have reported the tunnel pattern of many insects, proposed the process of nesting architecture by insects, and have opined that the dung beetles follow the

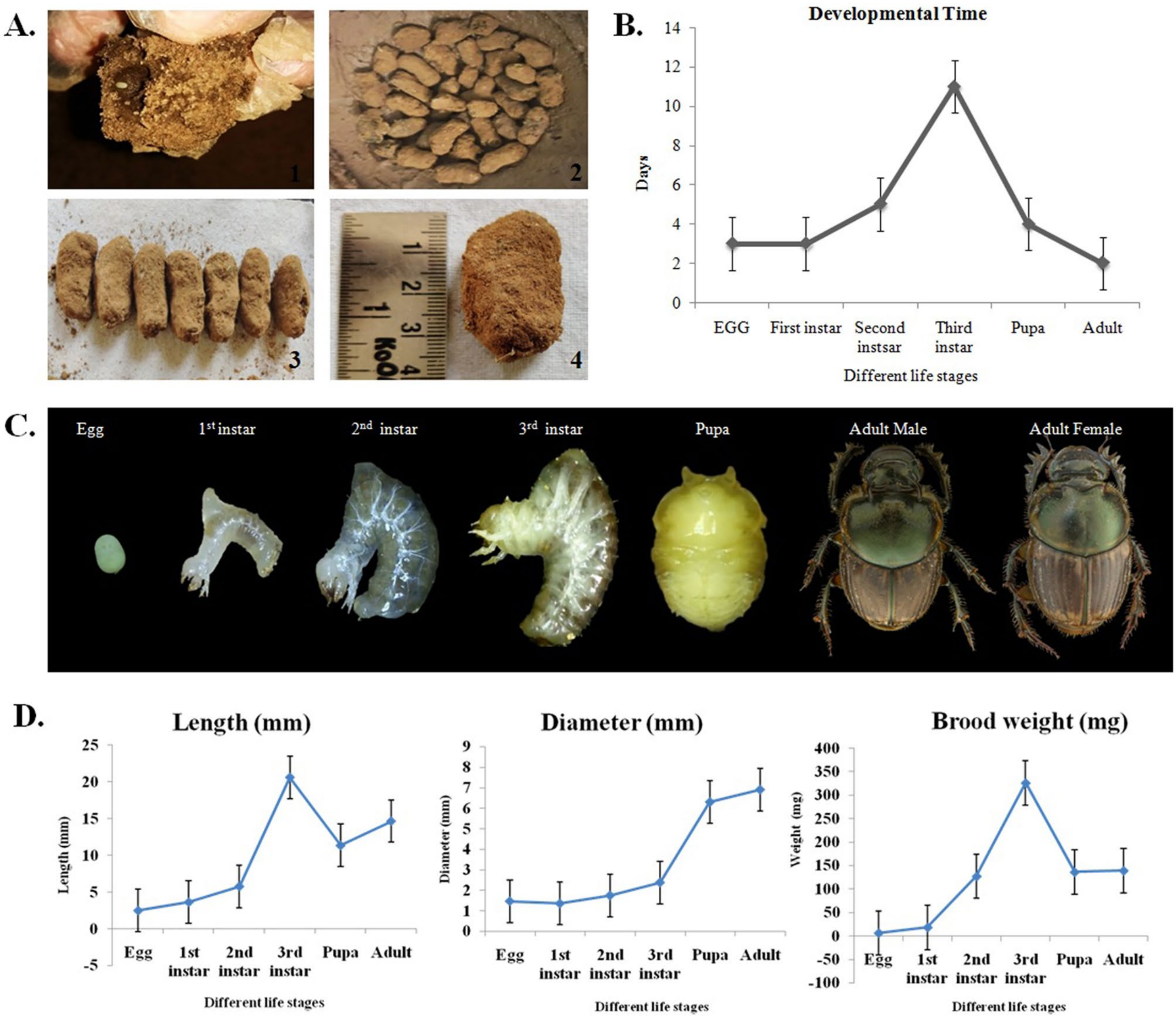


Fig. 4 Study on brood masses and life cycle of *D. gazella*. **A** Brood morphometry. Brood balls of almost similar measures were noted (2, 3, 4) and each brood ball contains one egg (1). **B** Comparative account on the duration of the different stages in the life cycle of *D. gazella*. **C** The stages of the life cycle starting from egg, larva (1st, 2nd, and 3rd instar), pupa and adult are observed. **D** Comparative account of brood length, diameter, and weight of different stages of the life cycle of *D. gazella*. Brood length, diameter, and weight increase with higher developmental stage except that the 3rd instar larva shows the maximum length and weight. Here, $n = 5$

process of Markovian-building as it helps them to construct a larger and deeper pit lined with steeper walls which protect the broods. Earlier, it has been reported that the width of the tunnel is directly proportional to the beetle's body size (Klingenberg and Monteiro 2005). However, in the present study, similar-sized dung beetles were selected and therefore no difference was observed in the length and diameter of the tunnel (Bertossa 2011; Macagno et al. 2016).

Tunnelers make nests and lay spherical, cylindrical brood masses by sexual co-operation. Once the egg is laid, female seals the broodball with dung and soil for protecting the growing larvae and pupa. As the brood turns into an adult, it comes out of the brood ball and undergoes sexual maturation (Huerta and García-Hernández 2013). In natural conditions, *D. gazella* digs a simple and deeper nest and forms several brood masses in a single tunnel (Moczek 2010; Hernández et al. 2011; Hanski and Cambefort 2014). Conversely, in the present study, although *D. gazella* dug deeper tunnel, the number of brood masses formed were not as high as described in natural conditions. The reduced number of brood masses is perhaps due to the restricted area provided in the laboratory conditions. However, the brood morphometry did not show any alterations, our observations are in accordance with the earlier work (Moczek 2010; Singh et al. 2019).

Dung beetle's larvae are known to continously restructure and physically modify their brood ball environment for the

Table 2 Brood morphometry of Different developmental stages (Mean ± SD) of *D. gazella*

Stage	Length (mm)	Diameter (mm)	Brood weight (mg)
Egg	2.49 ± 0.08	1.47 ± 0.09	6 ± 0.67
1st instar	3.63 ± 0.56	1.36 ± 0.06	18 ± 0.56
2nd instar	5.78 ± 0.94	1.75 ± 0.74	127 ± 0.83
3rd instar	20.64 ± 1.98	2.38 ± 0.56	326 ± 0.43
Pupa	11.36 ± 2.39	6.3 ± 0.83	136 ± 0.58
Adult	14.67 ± 1.78	6.9 ± 1.49	139 ± 0.16
Brood ball	33.72 ± 5.89	7.8 ± 0.89	745 ± 1.34

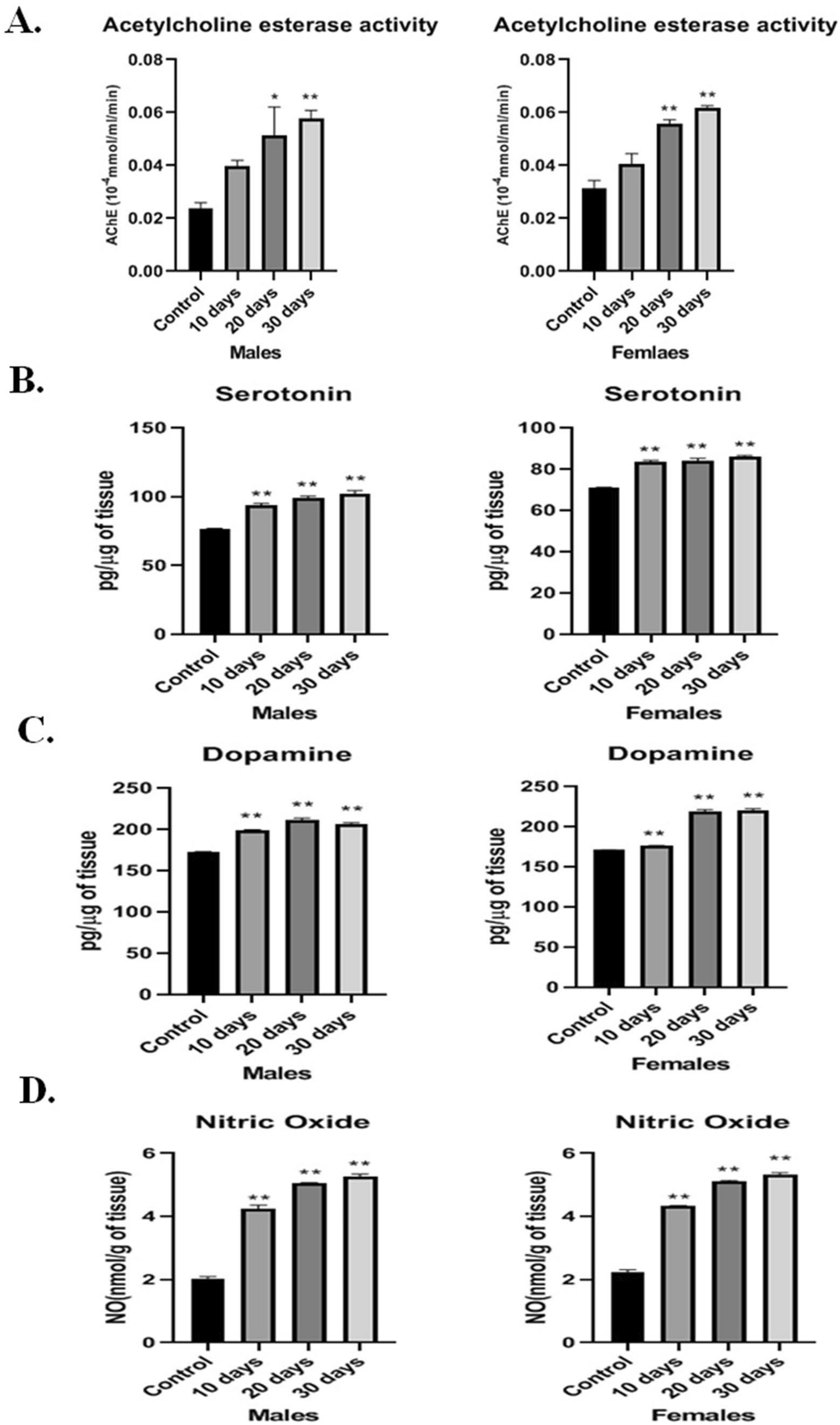
benefit of their growth and subsequent adult fitness. Previous research suggests that the relatively small *Onthophagus taurus* is considerably more dependent on such brood ball modifying behaviour than the much larger *D. gazella* (Schwab et al. 2017). In the present study, the brood ball morphometry was also observed throughout the life cycle, there was no difference in the morphometry of the brood ball. Thus, our observation are in agreement that *D. gazella* being larger in size compared to *O. taurus* does not restructure or modify the broodball environment (Kishi 2014; Rohner and Moczek 2021).

Neurotransmitters play a critical role in regulating many aspects of insect physiology and biochemistry. They also coordinate larval growth and maturation and ensure normal individual development (Di Bari et al. 2016; Trang and Khandar 2020). Previous studies have revealed that brain activity of AChE increases just after eclosion and remains at this stage throughout life in *Apis mellifera*, *Tribolium castenum*, *Drosophila melanogaster* (Hao et al. 2021). Further, precedent observations have revealed an apparent correlation between physical activity and levels of AChE in active insects such as houseflies, honeybees, ants, and cockroaches than in less active insects such as lepidopterous larvae (Grünewald and Siefert 2019). The increased AChE in adult male and female dung beetle accounted in the present study is thus self-explanatory and in agreement with the earlier work done (Palestrini and Rolando 2001). Further, a distinct difference in the level of AChE in males and females validates more physical activity for tunnel making and broodball formation.

Biogenic amines are neuroactive substances which controls responses of sensory neurons, activities of neurons, and movements of muscles, resulting in modification of behaviour (Watanabe and Sasaki 2021; Sasaki and Watanabe 2022). The ubiquitous biogenic amines, 5-HT and DA activate neural circuitry to regulate behaviour (Libersat and Pflueger 2004; Bergan 2015). Conserved aminergic circuits (Barron et al. 2010; Perry et al. 2016) and patterns of receptor expression (Blenau and Thamm 2011) control behaviour in diverse species across insect orders. The principle biogenic amines (DA and 5-HT) interact with hormone signalling pathways to elicit distinct behavioural and developmental responses (Pfaff and Joels 2016). Dung beetles show multiple occurrences of the evolution of familial sociality, including biparental care (Costa and Costa 2006; Cunningham et al. 2015; Panaitof et al. 2016). Burying beetles possess a astonishing neuroendocrine control with reference to their reproductive strategies, which includes tunnel pattern, broodball making, and parental care of their young ones (Hunt and Simmons 2002; Harano et al. 2008). In the present study in an attempt to have an insight for the role of biogenic amines, a significant elevation of DA and 5-HT in males and females on the 10^{th}, 20^{th}, and 30^{th} days of tunneling is implying the probable role of these neurotransmitters in nesting behaviour (Misof et al. 2014; Song et al. 2015; Kamhi et al. 2017). A marginal high titer of 5-HT in males compared to females (Trumbo 2019) can be correlated to the social context-dependent aggression in males during copulation (Stevenson and Schildberger 2013; Alekseyenko and Kravitz 2014). Parallel to the increase in 5-HT, an increased level of DA in males and females further reflects and proves the mitigating role of DA and its delicate adjustments in reinforcing the nesting activities (Rillich and Stevenson 2014; Guerra et al. 2016; Bhatt et al. 2018; Auletta 2019).

The insect olfactory system has evolved several modulatory systems to maximize foraging efficiency for resources. Foraging behaviour is reported to be maximally sensitive to olfactory cues in many insects (Kloppenburg and Mercer 2008; Mizunami et al. 2009; Verlinden 2018; Linn et al. 2020; Chatterjee et al. 2021). In insects, mating triggers changes in the behaviour and physiology of females, such as increasing oviposition and re-mating (Avila et al. 2011; Al-Wathiqui et al. 2016). Oviposition is known to be elicited by peptides and proteins transferred from male accessory glands through mating (Carmel et al. 2016) and by physical stimulation by males during mating (Li et al. 2020). The increased level of DA in the present study is thus in response to the olfactory stimuli as well as mating during the nesting.

Being a comparatively recently discovered neurotransmitter, the functions of nitric oxide in the nervous system are still only partially known. NO has been proved to promote habituation and has been implicated in modifying diverse neuronal circuits, such as increasing the digging rhythms of ovipositing and sensitivity of the taste receptors to chemicals (Cano et al. 2017). NO has been described to act as a retrograde neurotransmitter and plays an important role in reproduction, learning, and memory (Strauss 2002; Popov et al. 2005; Wessnitzer and Webb 2006; Ridgel et al. 2007). NO has a potent role in the signalling mechanism of the insect nervous system and participates by controlling behaviour at various levels such as perception of external stimuli, integration, selection of appropriate action and adaptive performance by neuron-muscular and neurosecretory systems


A.
Acetylcholine esterase activity
AChE (10^{-4}mmol/ml/min)
0.08
0.06
0.04
0.02
0.00
Control
10 days
20 days
30 days
Males
Acetylcholine esterase activity
Femlaes
B.
Serotonin
pg/µg of tissue
150
100
50
0
Males
Serotonin
100
80
60
40
20
0
Females
C.
Dopamine
250
200
150
100
50
0
Males
Dopamine
Females
D.
Nitric Oxide
NO(nmol/g of tissue)
6
4
2
0
Males
Nitric Oxide
Females

◂**Fig. 5** The levels of Neurotransmitters (as given in the Supplementary table II a, b, c) on the 10^{th}, 20^{th}, and 30^{th} day of introduction of males and females into the experimental setup. **A** Rate of AChE activity (with the unit mmol/ml/min $\times 10^{-4}$ per g of tissue). **B** 5-HT Levels. **C** DA Levels. **D** Nitric oxide content. The error bars indicate SE with significant values; $*p < 0.05$ $**p < 0.01$

(Heinrich and Ganter 2007; Weinrich et al. 2008). Foraging behaviour has been well explored in Drosophila larvae and *Bombyx mori* (Seki et al. 2005), experience-dependent fighting in crickets (Aonuma et al. 2004) and for sound production in grasshopper (Wenzel et al. 2005). In the present study, a significant increase in NO was observed in both male and female dung beetles which partially uncover the contribution of NO in above mentioned behaviour. However, a detailed analysis of the NO and its associated signalling molecules will help us in understanding and uncovering the exact role of NO in the dung beetles.

As this study was done only for *D.gazella*, we need to extrapolate it to the other species like *Heliocopris gigas* etc.which will help us to understand the species specific differences if any. Additionally, field based experimental regimes can be employed, that will throw more light in understanding the underlying phenomena *i.e.* the roles of neurotranmitters and extrapolating the obtained results in the controlled conditions. Thus, the findings will help us to understand the complexity of behaviour and will ultimately open up new dimensions for the further research.

Conclusion

Dung beetles provide several ecosystem services and are ideal bio-indicators since they are useful in studying the effects of urbanization or anthropogenic activities like habitat destruction, fragmentation, and edge effect on biodiversity. So, the conservation of dung beetles is necessary as the quality of these ecosystem services depends solely on their diversity, abundance and biomass. However, little is known about the reproductive strategies and the underlying neuroendocrine mechanism that individual body contains over such services. Hence, deeper understanding of the physiological basis of ecosystem services provided by dung beetles as well as individual-based perspective of *D. gazella* endows a better understanding on its ecology and biology. Therfore, the present study throws a light on the role of neurotransmitters in the nesting behaviour of dung beetle. It was observed that the reproduction pattern mainly depends on their tunnel pattern, which are habitually lined with dung, and they construct "brood balls" from dung where in they lay egg. The significant elevation of AChE, DA, 5-HT, and NO levels, in the brain of dung beetles on the 10^{th}, 20^{th} day, and 30^{th} day is intriguing to link neurotransmitters with nesting behaviour in *D. gazella*. Further, our findings suggest that of all the neurotransmitters, DA and 5-HT have a prominent role in the behavioural transitions associated with the initiation of tunnel patterns. To our knowledge, this is the first study exploring the link between neurotransmitters and the nesting behaviour of *D. gazella*. However, the molecular mechanism responsible for the upregulation of the neurotransmitters will further help us in understanding the exact role of neurotransmitters and is under investigation.

Abbreviations DA: Dopamine; 5-HT: 5- Hydroxytryptamine (Serotonin); AChE: Acetylcholine esterase; ACh: Acetylcholine; NO: Nitric oxide; CNS: Central Nervous System

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Author's contribution BT, PP, *PP contributed to the study's conception and design. NP, JP and MP collected all relevant publications and performed the experimental work. NP, BT, PP revised and formatted the manuscript. NP, PP carried out formal analysis and investigations. Original draft was prepared by NP, BT, PP. PP & *PP read, formatted and approved the final manuscript.

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RESEARCH ARTICLE

Molecular Characterization of Coleopteran Pests and its Relations to Agricultural Crops

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Abstract Coleopterans are adapted to a diversified range of environments and ecosystems. But there is a wide gap between systematic and genetic information of Coleopteran pests and their relation to agricultural crop hosts. Therefore, in the present research, an attempt was made to study the diversity of Coleoptera in agriculture fields of Vadodara district, Gujarat. The study sites (Ajwa, Channi, Karjan and Padra) were visited twice a month and sampling was carried out twice a day (dawn and dusk) for 2 years (August 2017–2019). Morphological as well as molecular identification using DNA sequencing of COI and 16srRNA was done. A total of 69 Coleopteran species belonging to 16 families were collected, and barcodes of 16 species were successfully submitted to Gene bank. Further, phylogeny was resolved using Neighbour-Joining cluster and Maximum Likelihood analysis methods, and concordant results were obtained: Chrysomelidae was found closest to Cerambycidae, while Curculionidae was closest to Meloidae, and Scarabaeidae with Elateridae. The barcoding of interactions between insect pests and agricultural crops were also performed with COI and trnL markers, where species-specific homology modeling accurately deciphered the phylogeny and unraveled intraspecies and interspecies divergence nucleotide distance.

Keywords Coleopteran pests · Host-pest interaction · Molecular markers · Species-specific pest-crop association

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Introduction

Insects are taxonomically diverse group having a wide-ranging distribution and a complex evolutionary history (Sahney et al. 2010; Speight 2017). They are renowned in areas of agricultural pests, major disease vectors, pollinator of crops, parasites of other insects, and bio-indicator of environmental changes (Price et al. 2014; Mandal et al. 2014; Bouchard et al. 2017). With an estimated 1.5 million different species of beetles, Coleoptera make up over 40% of all described arthropod species (Stork et al. 2015).

The taxonomical studies of any organisms are a challenging mission and require experts in the concerned field. The study often involves complicated procedures and it is time-consuming. It does not always provide resolution at the species level (Pentinsaari et al. 2014; Behura 2015; Karthika et al. 2016). The DNA barcoding, the nucleotide-based taxonomic classification, is found to be helpful in the taxonomical identification of species (Navarro et al. 2010; García-Robledo et al. 2013; Syfert et al. 2017). The determination of phylogenetic relationships of organisms is done through the combination of the morphological and molecular analysis based methods. The phylogenetic studies by using molecular markers are important as well as dominant

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INSECTICIDAL ACTIVITY OF ESSENTIAL OILS FROM MINT AND AJWAIN AGAINST PULSE BEETLE *CALLOSOBRUCHUS CHINENSIS* (L)

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ABSTRACT

The present study evaluates the insecticidal activity of two essential Oil (EOs) mint (*Mentha arvensis*), and ajwain (*Carum capyicum*) against pulse beetle (*Callosobruchus chinensis)* (L). Contact toxicities of these were evaluated using parameters of lifecycle like total development period, numbers of eggs laid, adult emergence and adult longevity. Along with these detoxification enzyme inhibition activities of acetyl cholinesterase (AChE), alkaline phosphatase (ALP), transaminases enzymes- aspartate aminotransferases (AST) and alanine aminotransferases (ALT) and total protein were estimated. EOs were observed showing toxicity (mint LC_{50} = 5.9 µl/ ml and ajwain LC_{50}= 7.02 µl/ ml). Exposure of EOs altered the lifecycle parameters significantly ($p<0.01$). The detoxification enzyme inhibition activities were also significant ($p<0.01$). Thus, it is concluded that these EOs can be recommended as safe and ecofriendly alternatives.

Key words: *Callosobruchus chinensis*, essential oils, *Mentha arvensis*, *Carum capyicum*, lifecycle, acetyl cholinesterase, alkaline phosphatase, transaminases enzymes, inhibition

India is one of the leading producers of food in the world and it produces more than a billion tonnes of agricultural product. 58% of India's population is dependent on agriculture as its primary source of livelihood. In India, advancement of technology has increased the production of grains; however, improper storage has resulted in huge loss and has been reported to be around INR 926 billion loss annually (Singh and Khanna, 2019; Sirohi et al., 2021). Infestation of stored grain by many insects, mite and fungi degrade the quality and quantity of grains (Lal et al., 2017; Jerbi et al., 2021). The total productivity of agricultural crops of India is 3 tonnes/ha; out of which loss due to insect pest is about 26 % (Lal et al., 2017), like the lesser grain borer, *R. dominica*'s larva and adult infests the grains and declines its quality (Jerbi et al., 2021). Rice pest *S. oryzae*, causes qualitative and quantitative loss (Saad et al., 2018). *C. chinensis* a major pest of stored pulses and is reported to cause 32-64% loss under storage condition (Femeena et al., 2018). After discovery of DDT, Insect pests are mainly controlled by synthetic pesticides (Lal et al., 2017; Demeter et al., 2021). WHO has reported that every year two lakhs people die due to pesticide poisoning owing to its carcinogenic and teratogenic properties (Sarwar, 2016). Use of synthetic pesticide is a easy and quick solution for controlling insect pests but pose a potential risk not only to humans but also to the environment as their residues have been reported to be present in soil, air and water (Said and Pashte., 2015; Lal et al., 2017). The repeated uses of synthetic insecticide for decades has disrupted biological control by natural enemies and has led to outbreaks of other insect species and at times have resulted in resistance of pesticides in insect pest (Hill et al., 2017; Hawkins et al., 2019). Hence, there is need for alternative solution which environment friendly does not harm other non-target species. Plants and their derivatives have been proved to be a viable alternative as more than 2000 plant species have been recorded to possess insecticidal properties and possess low health risks (Pavela, 2016; Jerbi et al., 2021). EOs are naturally produced by plants as secondary compounds which are volatile, but as natural products protects the stored grains from pest attack (Omar, 2020). EOs has multiple components mixture and causes toxicity by interfering with various aspects of insect's physiology and biochemistry (Kiran et al., 2017). Present work evaluates the insecticidal potential of the two EOs *M. arvensis* and *C. capyicum* against *C. chinensis* (pulse beetle) adults.

MATERIAL AND METHODS

The adult insects were collected from the infested grains from the granary and were reared on 500 g green gram (variety - Sabarmati PS 16) maintained in laboratory at Department of Zoology, The Maharaja Sayajirao University of Baroda. A culture of *C. chinensis*

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ECOLOGICAL ROLE OF *ONTHOPHAGUS TAURUS* (SCHREBER) IN SOIL NUTRIENT MOBILIZATION

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ABSTRACT

Dung beetles play a major role in the pasture ecosystem. The manure recycling activity of dung beetles is linked to their tunneling behavior. The present study was designed to analyze the tunnel pattern and nutrient mobilization by dung beetles, *Onthophagus taurus* (Schereber, 1759) in different soil types. A simple type of tunnel pattern was observed in all the four types of soil after 30th day of their introduction (10 pairs of male and female) into the experimental setup. However, the maximum number of tunnels was observed in the sandy and sandy clay loam (no. of openings- 15), followed by loamy soil (no. of openings- 13). The physical (texture, water holding capacity, porosity, moisture content) and chemical parameters (pH and nutrients) of all the four types of soils were evaluated. Soil texture analysis revealed the texture to be of sandy (yellow soil), sandy clay loam (red and black soil), and loamy sand (brown soil) types. Water holding capacity and the soil porosity were recorded highest in the sandy soil, whereas moisture content was found maximum in the sandy clay loam. Soil nutrient analysis illustrated a significant increase in the amount of nitrogen (N), phosphorus (P), calcium (Ca), sulfur (S), sodium (Na), potassium (K), organic carbon and organic matter. Thus, the present study confirms that tunneling activity of *O. taurus* enhances the soil nutrients by carrying out dung decomposition.

Key words: Dung beetle, *Onthophagus taurus*, nesting, tunneling, nutrients, soil parameters, texture, water holding capacity, porosity, moisture, nutrients, sandy, clay, loam, red and black soils

Arthropods are one of the most successful and cosmopolitan group of animals on earth. Their ability to adapt to the changing environment makes them the most successful and diverse group of animals (Giribet, 2019). Among the arthropods, class Insecta is the largest group and the order Coleoptera is the leading order of the animal kingdom constituting almost 25% of all the living organisms and it includes around 3,50,000 species worldwide and among these around 15,088 species are present in India. Among 25% of insect species, 40% are beetles (Thakkar, 2016). Scarab beetles commonly known as dung beetles of the family Scarabaeidae have approximately 30,000 species of beetles (Cajaiba et al., 2017). They exhibit a wide range of ecological, morphological as well as behavioral adaptations which makes them universally distributed. Mostly dung beetles prefer to be omnivore, than herbivore dung, and the least preferred is carnivore dung (Frank et al., 2017, unpublished data). Mandibles and maxillae of adult dung beetles have a fine outer edge which helps in modifying and filtering out the content of dung (Shukla et al., 2016). Further, tibia of forelegs have spines and spurs which helps them in digging and forming the tunnel. Tibial spur number varies among the species which helps taxonomist to classify the dung beetles (Linz et al., 2019). In addition, head of the dung beetles has a hard, scoop like structure which helps in rolling the dung balls for their nesting (Ix-Balam et al., 2018). *Onthophagus taurus* (Schreber), as a tunneler makes "multimedia galleries" (tunnels) deep into the soil for laying eggs in the brood balls. These tunnels can be formed by both male and female or only by single parent. Brood balls are placed into the blind end of the tunnel. Single branch of these complex tunnels may contain one or multiple brood balls (Tonelli, 2021). This behavioral aspect enhances their ecological efficiency for dung decomposition, bioturbation, seed dispersal, parasite suppression, fly control and nutrient recycling (Shahabuddin et al., 2017). Further, tunneling activity makes the continuous movement of the soil and thereby increases soil aeration and its water holding capacity (Nichols et al., 2008; Doube, 2018). Dung produced by livestock are source of many greenhouse gases such as nitrous oxide (N_2O), methane (CH_4), and carbon dioxide (CO_2) which is reduced by dung beetles by reducing organic matter from the dung by their relocation into the soil (Piccini et al., 2017).

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RESEARCH

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Evaluation of insecticidal potential of organochemicals on SF9 cell line

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Abstract

Background: Organophosphates and Pyrethroids are the most widely used pesticides worldwide and are known to have significant toxicity on the nervous system of the target pest. Assessment for combined toxicity of Organophosphate and Pyrethroid on Sf9 (*Spodoptera frugiperda*) cells is less explored. The present study demonstrates and compares the two organochemicals whose trade names are Ammo and Profex, for its cytotoxic potential on the insect Sf9 cells. Ammo and Profex were selected as the test chemicals as toxicity of these insecticides at molecular and cellular level is poorly understood.

Results: The results of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide assay demonstrated that Ammo and Profex exhibited significant cytotoxicity to Sf9 cells in a time- and dose-dependent manner. In our study, the IC_{50} value was obtained by MTT assay and the sub-lethal concentrations ($IC_{50}/_{20}$-17.5 μg/ml, $IC_{50}/_{10}$-35 μg/ml, and $IC_{50}/_{5}$–70 μg/ml for Ammo and $IC_{50}/_{20}$-20 μg/ml, $IC_{50}/_{10}$-40 μg/ml, and $IC_{50}/_{5}$-80 μg/ml for Profex) were selected for further tests. Acridine orange/ethidium bromide staining proved the apoptotic cell death on exposure of both the insecticides confirming its toxic potential. Furthermore, antioxidant status was assessed using DCF-DA staining and both the insecticides resulted into an increased reactive oxygen species (ROS) generation. A dose- and time-dependent significant ($p < 0.05$) alterations in lipid peroxidase (LPO), glutathione (GSH) and catalase (CAT) activity were observed.

Conclusion: The results showed that both Ammo and Profex triggered apoptosis in Sf9 cells through an intrinsic mitochondrial pathway via the generation of ROS. Of the two insecticides, Ammo was found to be more toxic compared to Profex. The present study is important to evaluate the environmental safety and risk factors of Organochemicals' exposure to crops and livestock.

Keywords: Sf9 cell line, Organophosphate, Profex, Ammo, Apoptosis, ROS, LPO, GSH, CAT

Background

Organophosphate (OP) insecticides are among the most common class of pesticides that are mainly used to control the insect pest populations. They are the group of insecticides whose key target is to inhibit Acetylcholine esterase (AChE) which is responsible for hydrolysis of Acetylcholine. The OPs phosphorylate the hydroxyl group of a serine residue on AChE in the central nervous system. It has been reported that excessive use of these insecticides in the public health and agriculture leads to environmental pollution causing a number of acute and chronic poisoning events (Lukaszewicz-Hussain, 2010). However, the prolonged use of insecticides has been known to reduce its effectiveness among the target insect pests. Thus, the need to search for novel insecticides with better efficacy or a new mode of action becomes evident.

Nowadays, mixed pesticides are in great demand for agricultural use because of their efficiency, convenience, and rapid actions (Zhou et al., 2011). One of the pesticides widespread in use is the combination of Organophosphate and Pyrethroid due to its low mammalian

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Chapter

6

Nesting Pattern and Soil Nutrient Enrichment by Dung Beetles (Coleoptera: Scarabaeidae): An Ecological Approach

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ABSTRACT

Dung beetle species compete for dung and the resources are utilized for food and nesting for adults and larvae. The development of the adult depends mainly on the quantity and quality of resources consumed during the larval period. During parental care, they show preference for dung of greater nutritional quality, which in turn, impact their assemblage and tunneling behaviour. In the present study an attempt is made to explore the dung preference in three species of Scarabaeinae: *Onthophagus taurus (O. taurus)*, *Digitonthophagus gazella (D. gazella)*, and *Heliocopris gigas (H. gigas)* from Vadodara district, Gujarat. Of all the selected dungs, Buffalo (*Bubalus bubalis*) and Cow dung (*Bos indicus*) was the most preferred dung. Further, biomolecular estimations were performed to check the nutritional content of the dung, which revealed that amongst all, carbohydrate content was highest in the dung of Cow (0.018mg/ml) and Bufffalo (0.0137mg/ml). The nesting pattern of all the tunnelers showed a distinct pattern which is probably dependent on the body size of the beetle. *O. taurus* and *H. gigas* formed a complex nest while *D. gazella* made a simple nest. In addition, to evaluate the ecological role of the dung beetles, an attempt was made to check the nutritional enrichment of the soil, and it was reported that *H. gigas* and *D. gazella* significantly ($p<0.05$) increased the inorganic content: Nitrogen (N), Phosphorus (P), Potassium (K), Calcium (Ca) and Magnesium (Mg) as well as organic carbon and organic matter into the soil. These varied patterns of consumption and relocation of dung by beetles drive a series of ecological processes such as nutrient cycling, soil aeration, and secondary seed burial. Hence, it can be concluded that the ecological role of the dung beetles is well dependent on the nutritional content of the dung for performing their beneficial services to the agroecosystem.

Keywords: Dung, Dung Beetles, Scarabaeidae, Tunnel Pattern, Ecological Role, Nutritional Content.