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Effect of biotic and abiotic factors on the abundance of greater wax moth, *Galleria mellonella* L.

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Abstract
Galleria mellonella, a commonly found nuisance for honey bees in regions where beekeeping is practiced, predominantly poses a threat through its larval stage. These larvae consume beeswax, honey, pollen, and the pupal remains of bees, causing destructive impacts. The population of *G. mellonella* tends to peak between April and July, gradually declining until March, with the highest numbers recorded in July. Numerous factors contribute to its abundance, including both living (biotic) and non-living (abiotic) elements. Biotic factors involve early instars engaging in cannibalism, the type and nutritional content of food, the presence of parasitoids like *Bracon hebetor* and *Dibrachys cayus*, and the targeted bee species. Abiotic factors encompass fluctuating temperatures, relative humidity, and varying daylight durations. This research aims to delve deeply into the biology of the greater wax moth and explore the influence of diverse biotic and abiotic factors on their population, along with correlation studies.

Keywords: *Galleria mellonella,* Seasonal incidence, biotic factors, abiotic factors

Introduction

Greater wax moth, *Galleria mellonella* is a species of moth, belonging to family pyralidae of order Lepidoptera, often used in scientific research (Wojda *et al*., 2020) [99]. These moths have fascinating characteristics and is a seriously destructive pest of honey bee, both in storage and apiary. It can be found anywhere beekeeping is practiced (Williams, 1990; Shimanuki, 1971) [96, 83]. Its origin traces back to Asia, and then it spread to Northern Africa, Great Britain, some parts of Europe, northern America, and New Zealand (Akratanakul, 1987; Paddock and Floyd, 1918) [2, 71]. *Galleria mellonella* is a pest of different honey bee species *viz., Apis mellifera, A. cerana, A. dorsata* and *A. florea* (Hood *et al*. 2003) and even the stingless bees (Nogueira-Neto, 1997) [66] and the bumble bees (Oertel, 1963) [67]. They cause destruction to the bees by feeding on wax while burrowing through the midrib of the wax combs and at the same time, by transmitting different bee viruses like the deformed wing virus (DWV) and black queen cell virus (BQCV) (Kwadha *et al*. 2017) [52]. Apart from the field of entomology they are also useful in studying various fields like immunology, microbiology, and even genetics (Wojda *et al*., 2020) [99]. Researchers commonly employ them as a model organism due to their ease of handling, short life cycle, and their ability to host various pathogens (Killiny, 2018) [44]. *Galleria mellonella* acts as a model organism to study in-vivo toxicology and pathogenicity (Ignasiak and Maxwell, 2017) [34]. It is used to study the interactions of entomopathogenic biopesticides like *Beauveria bassiana, Bacillus thuringiensis* (Ruiu, 2015; Ortiz-Urquiza *et al.* 2015) [79, 69]. The pest larvae are also used to study virulence of certain human pathogens too such as *Pseudomonas aeruginosa, Enterococcus faecalis, Staphylococcus aureus, Candida albicans, Fusarium oxysporum,* and *Aspergillus fumigatus* (Maekawa *et al.* 2015; Koch *et al.* 2014; Munoz-Gomez *et al.* 2014; Gomez-Lopez *et al.* 2014; Gibreel and Upton, 2013) [57, 46, 62, 26, 25]. They've contributed significantly to our understanding of host-pathogen interactions and immune responses. They are used in the identification and evaluation of novel antimicrobial agents, thus replacing the use of mammals in several experiments (Piatek *et al.* 2021; Harding *et al.* 2013) [75, 31]. Vijaykumar *et al.* 2019 [92] reported that *G. mellonella* was responsible for 60-70% of economic loss in beekeeping in India.

The *G. mellonella* have four life stages *viz*., egg, larva, pupa, and adult (Desai *et al*. 2019; Jorjão *et al*. 2018; Kumar and Khan, 2018; Hosamani *et al*. 2017; Kwadha *et al*. 2017) [21, 42, 33, 51, 52]. The adult attacks the weaker colonies with a population less than 20000 bees or stored combs at night. They lay the eggs in the cracks and crevices of the hive (Vijaykumar *et al*. 2019) [53]. The larvae hatch out and start feeding the wax combs (Britannica, and Editors of Encyclopaedia, 2017) [12]. They primarily feed on the midrib of the comb and form galleries lined with silken threads. The emerging bee gets entangled in those silk and starves to death, leading to a condition called galleriasis (Kwadha *et al*. 2017; Gulati and Kaushik, 2004; Williams, 1997) [52, 29, 97]. As the cells are fed by the larvae, it affects honey bee by emergence of deformed adults or dead adults entangled in silk. Apart from wax, they also feed on pollen, and bee castings (Hosamani *et al*. 2017; Kwadha *et al*. 2017) [33, 52]. The continued infestation of the hive may lead to absconding, colony loss and lower bee swarms, if proper steps are not adopted to control it (Kwadha *et al*. 2017; Gulati and Kaushik, 2004; Williams, 1997) [52, 29, 97].

Seasonal Incidence

Seasonal changes significantly influence the life cycle and behavior of *Galleria mellonella*, commonly known as the greater wax moth larvae. These insects demonstrate fluctuations in both their population size and activity levels in response to seasonal variations. Oliveira *et al*. (2018) [68] highlighted distinct seasonal shifts in *G. mellonella* populations, showing higher prevalence during warmer months and reduced occurrences in colder seasons. The moth population typically reaches its peak during warmer months and diminishes as temperatures decrease. Jia *et al*. (2020) [37] investigated the seasonal dynamics of *G. mellonella* and noted heightened population density during spring and summer, correlating with optimal temperatures and environmental conditions conducive to their growth and reproduction.

Lalita *et al*. (2022) [53] conducted research at Chaudhary Charan Singh Haryana Agricultural University in India, revealing the highest numbers of *G. mellonella* moths in Apis melifera hives during July, with the lowest counts in March. They observed a consistent pattern of population increase from April to July followed by a decline toward March. Similarly, Bhatnagar *et al*. (2020) [8] observed minimal or no infestation of *G. mellonella* on Apis melifera in February and March, with the first noticeable increase in April and a subsequent rise in population until July. The adult *G. mellonella* population within the A. mellifera hive peaked from May to November, with August identified as the period of maximum abundance (Sohail *et al*., 2017) [89].

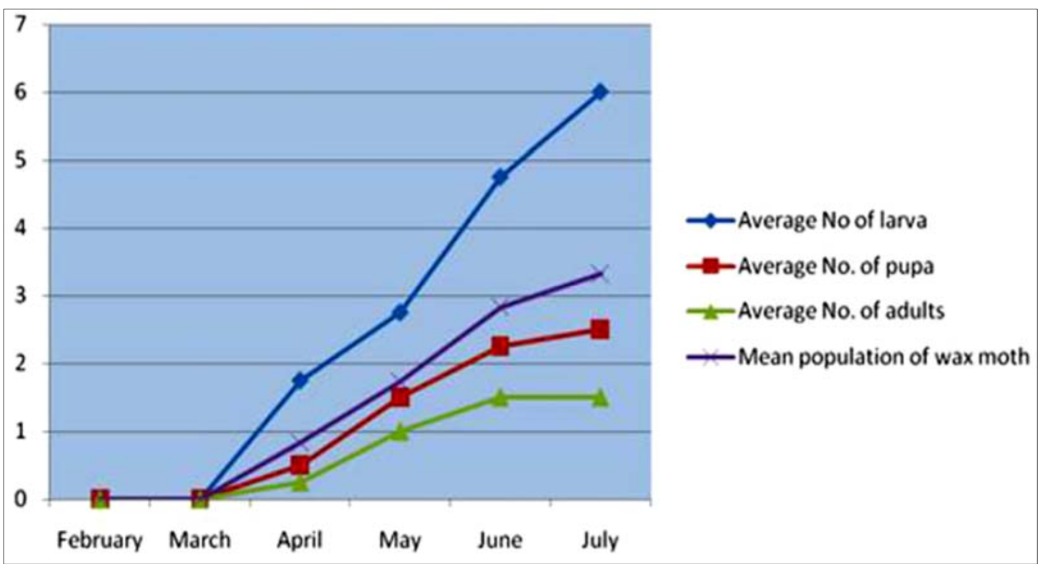


Fig 1: Seasonal incidence of Galleria mellonella stages (Bhatnagar *et al*. 2020) [8]

The peak larval infestation was observed in September, as documented by Lalita and Kumar (2022) [53], Yadav and Kumar (2022) [100], Sohail *et al*. (2017) [89], Mandal and Vishwakarma (2016) [81], and Indu *et al*. (2008) [35] concerning A. mellifera. Investigating A. dorsata, Raghunandan and Basavarajappa (2014) [77] highlighted the highest infestation rates in semi-arid regions during summer (30.8%) and the rainy season (23.4%). Comparatively lower rates were noted in the malnad region during summer (11%) and winter (6.6%), while notably reduced infestations were observed across all seasons in arid regions. Additionally, Lalita and Kumar (2022) [53] documented the highest count of larvae per comb, noting ten combs per hive, followed by 9, 8, and 7 combs in descending order.

Factors affecting the abundance of *Galleria mellonella L.*

As observed in the section above, the population of Greater wax moth varies throughout the year. This is because of several biotic and abiotic factors like Cannibalism, Food, Parasitoids, Host species, and Temperature, Relative humidity, and light respectively, affecting the growth of the moth's life stages (Nielsen and Brister, 1979; Charriere and Imdorf,1999) [65, 16].

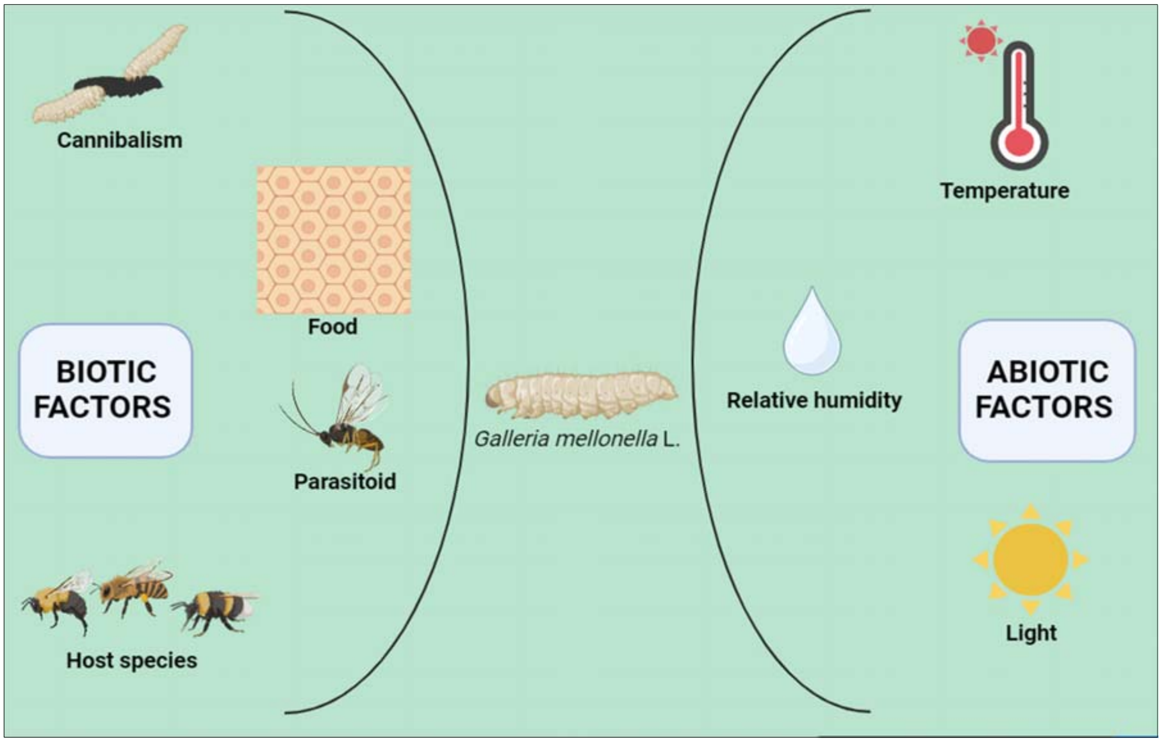


Fig 1: Abstract regarding the factors affecting the growth of Galleria mellonella.

The biotic and abiotic factors are known to regulate arthropod attraction, colonization, and utilization of decomposing vertebrate remains (Jordan and Tomberlin, 2017) [41]. They are discussed as follows:

A. Biotic factors

Biotic factors include the living organisms having their impact on the abundance of *G. mellonella* population. Living organisms interact with biotic factors such as food, natural enemies, prey, hosts, and have different inter and intra species interactions, that have an impact on their growth and reproduction. Lately, it was accepted that abiotic factors are the primary factors in determining the distribution of biodiversity, and the biotic factors were important at finer scales (Lewis *et al.* 2017; Wiens, 2011; Benton, 2009; Pearson and Dawson, 2003) [55, 95, 7, 73]. Biotic factors form an important base for the species distribution at broad spatial scales (Leach *et al.* 2016; Wisz *et al.* 2013; Van der *et al.* 2010; Meier *et al.* 2010; Guisan and Thuiller, 2005) [54, 98, 91, 58]. There can be interspecific and intraspecific factors that affect the developmental duration and the survival of *G. mellonella.* Intraspecific factors arise among the members of *G. mellonella* like competition for food (Williams, 1997; Nielsen *et al.* 1979) [97, 65], cannibalism by the later instar larva on the early instars and pupa (Kwadha *et al.* 2017; Nielsen *et al.* 1979) [52, 65]. The quality of its diet can also impact its development (Kwadha *et al.* 2017; Krams *et al.* 2015; Awmack and leather, 2002) [52, 3]. Interspecific factors can include interaction with parasitoids, Bracon *hebetor*, *Dibrachys cayus* etc. and the interaction with host species like *Apis melifera, A. florea, A. dorsata, A indica* etc. (Paddock and Floyd, 1918) [71].

i) Cannibalism

Insects of many feeding guilds that typically are not carnivorous also can be cannibalistic, including species that feed on plants, algae, bacteria, and detritus, or species that are omnivorous (Richardson *et al*., 2010; Crespi 1992) [78, 20]. It has been reported in around 130 non-carnivorous insect species (Richardson *et al*., 2010) [78]. Cannibalism is an intraspecific competition, observed in the larval stage during the process of moulting, when food is scarce (Kwadha *et al.* 2017; Serrano *et al*., 2023) [52, 82]. Nielsen and Brister, 1979 [65] reported cannibalism of the early larval instars and the pupal stages by the later instar larvae. Cannibalism is found only in case of shortage of food (Ménard *et al*., 2021; Kumar and Khan, 2018; Kwadha *et al.* 2017; Ben, 1999; Williams, 1997; Nielsen and Brister, 1979) [59, 51, 52, 97, 65]. Cannibalism has also been reported in crowded conditions, when larvae cannot enlarge their tunnels without disturbing other larvae (Nielsen and Brister, 1979; Williams, 1997) [97, 65].

ii) Food

Nutritional quality of food can have an intense effect on life and ability to fight and resists any kind of infection (Povey *et al.* 2013; Jimenez-Cort´es *et al.* 2012; Ayres & Schneider, 2009; Siva-Jothy & Thompson, 2002) [76, 46, 4, 87]. The larvae of *G. mellonella* feed on wax (and other impurities present in the beeswax), honey, pupal skins, pollen (Kwadha *et al.* 2017) [52]. Beeswax has elements like protein and vitamins for growth and development (Kandel *et al.* 2020) [43]. The larval diet impacts based on the level of cholesterol and protein in it (Htet and Ueno, 2019). A weak diet can result in a longer life cycle, a short ovipositional period, and a long egg incubation period (Mohamed *et al.* 2014) [60]. Deprivation of *G. mellonella* larvae of food leads to a reduction in the cellular and immune responses and an increased susceptibility to infection (Banville *et al*., 2012) [5]. The use of natural and artificial diet resulted in survival rate of 0.90 and 0.82, and sex ratio (female/total) of 0.67 and 0.51 (Kandel *et al.* 2020) [43] respectively. A study was conducted by Dadd, 1964 concluded that linoleic or linolenic acid was important for the successful emergence of the adult moths from the pupal stage. Also concluded that when casein was used as the only source of amino nitrogen, the growth was best. The use of new comb and old comb also impacted with the lowest (392 eggs, 5.2 days) and highest (1308 eggs, 8.4 days) fertility and oviposition periods (Mohamed *et al.* 2014) [60]. Kandel and colleagues (2020) [43] found that the time it took for the immature stages to develop was quicker when raised on wax frames (47.26 days) compared to being fed a laboratory diet of *G. mellonella,* which took about 57.5 days. However, they didn't detect any notable distinctions in the lifespan of adult females raised on these two diets.

Galleria mellonella is also reported to consume polystyrene and polyethene (Lou *et al.* 2020; Bombelli *et al.* 2017; Yang *et al.* 2014; Kim *et al.* 2014; Brenner *et al.* 1986) [56, 10, 101, 45, 11]. Bacteria of *Bacillus spp.* is found in higher rate of polythene consuming pests (Cassone *et al.* 2020; Lou *et al.* 2020) [15, 56]. The presence of phagosomes in larval hemocytes and mid-gut cells during PELD (Polyethylene low density) -only feeding suggested active involvement in plastic breakdown (Elmekawy *et al*., 2023) [23]. Ruiz *et al.* 2022 [80] reported that larvae consuming polystyrene and polythene completed their lifecycles in all the treatments. They also found polythene feeding larva transformed into pupae in significantly less time.

iii) Parasitoids

Bracon hebetor, a commonly found parasitic wasp within the Pyralidae family, has been identified as a significant parasitoids in stored insect pests. Adly and Marzouk (2019) [1] highlighted its potential for parasitizing fifth-stage larvae of *Galleria mellonella* in various settings such as laboratories, beehives, and stored combs in Egypt. The females of this parasitoid species immobilize host larvae through venom injection, specifically targeting glutamatergic receptors on the presynaptic membranes (Walther Rathmayer, 1974) [93], inducing paralysis. Moreover, this envenomation process significantly diminishes both the cellular and humoral immunity of the hosts, notably in wax moths (Kryukova *et al*., 2011; Kryukova *et al*., 2015) [50, 49].

Dibrachys cayus, also known as the Chalcid wasp, similarly parasitizes *G. mellonella* (Gounari *et al.* 2021) [27]. However, it primarily targets wax moth cocoons rather than the mobile larvae, preferring to lay more eggs when more wax moth cocoons are available. Notably, their research indicated lower parasitisation rates during the warmer period from June to August, with higher rates observed in other months.

iv) Host species

Hosamani *et al.* 2017 [33] didn't report any differences in pre-oviposition period for females significantly. However, the significant difference was noticed in the durations of oviposition and post-oviposition when reared on four types of combs, and slight differences in the incubation period, larval period, pre pupal and pupal period, and the longevity of the adults based the different bee species as its host. The period before egg laying, known as the preoviposition period, was longest, lasting 1.10 days on both A. cerana and A. dorsata. On A. dorsata, the time dedicated to egg laying (oviposition) and the period after egg laying (post-oviposition) were notably longer, spanning 5.00 and 1.30 days, respectively. There were significant differences observed in the reproductive capacity of females raised on combs from four honeybee species. Specifically, females reared on A. dorsata combs exhibited the highest fecundity, averaging 869.90 eggs per female, equivalent to 173.96 eggs per day.

Galleria mellonella prefers to attack the weak bee colonies, and in case of strong colonies, they attack during the reduction in population of the colonies (Bhatnagar *et al.* 2020; Swamy and Rajagopal., 2005) [8, 90].

Table 1: Effect of different bee species over the life cycle of Galleria mellonella (Hosamani *et al*. 2017) [33]

Different combs of honey bee species	Egg period (days)	Larval period (days)	Prepupal period(days)	Pupal period (days)
A. cerana	8.60 ± 0.52 (8-9)	49.30 ± 1.66 (47-53)	2.10+0.55 (1-3)	8.60+0.75 (8-10)
A. dorsata	8.60 ± 0.52 (8-9)	54.20 ± 1.32 (52-57)	2.35+0.49 (2-3)	9.40-0.60 (8-10)
A. mellifera	9.20 ± 0.63 (9-11)	110.65 ± 8.28 (91-123)	2.60+0.68 (2-4)	10. 05+0.69 (9-12)
A. florea	8.70 ± 0.48 (8-9)	58.60 + 3.03 (54-64)	2.40-0.50 (2-3)	9.65+0.75 (9-11)

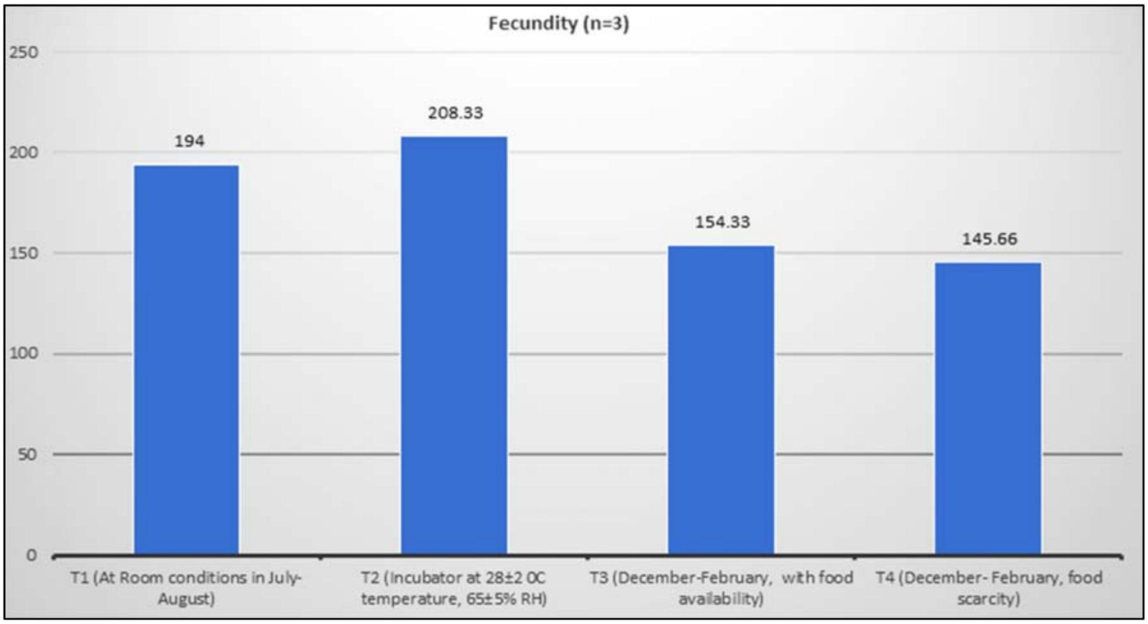
B. Abiotic factors

Critical abiotic elements essential for *G. mellonella ‘s* complete life cycle encompass temperature, humidity, and light. These factors have demonstrated influence on both the life cycle of *G. mellonella* and its susceptibility to infection (Pereira & Rossi, 2020) [74].

Wax moths are nocturnal insects that thrive in dark, warm, and poorly ventilated areas like beehive. Therefore, the most favorable environment for rearing the wax moths is approximately 30 °C, 70% relative humidity, and darkness (Warren and Huddleston, 1962) [94].

Typically, *G. mellonella* thrives within a temperature span of 25-37 °C. Maintaining relative humidity above 33% is crucial, as levels below this threshold decrease adult lifespan and reproductive capacity, hindering egg-laying abilities (Hanumanthaswamy *et al*., 2013) [30]. These specific temperature and humidity ranges align closely with the insect's survival regions globally (Williams, 1997; Shimanuki, 1981; Shimanuki *et al*., 1980) [97, 84, 85].

Moreover, light exposure plays a role in larval development, with constant illumination leading to a slowdown in larval metamorphosis and reduced final weight (Kryspin *et al*., 1974) [48]. Being a nocturnal insect, *G. mellonella* exhibits peak activity between 6:00 PM and midnight, primarily during the initial stages of darkness (Kwadha *et al*., 2017) [52]. Nielsen and Brister (1977) [64] observed that recently emerged male and female moths tend to fly toward nearby trees at nightfall, presumably for mating purposes. However, only females carrying spermatophores, containing eggs, return to honeybee colonies from these trees (Nielsen and Brister, 1977) [64].



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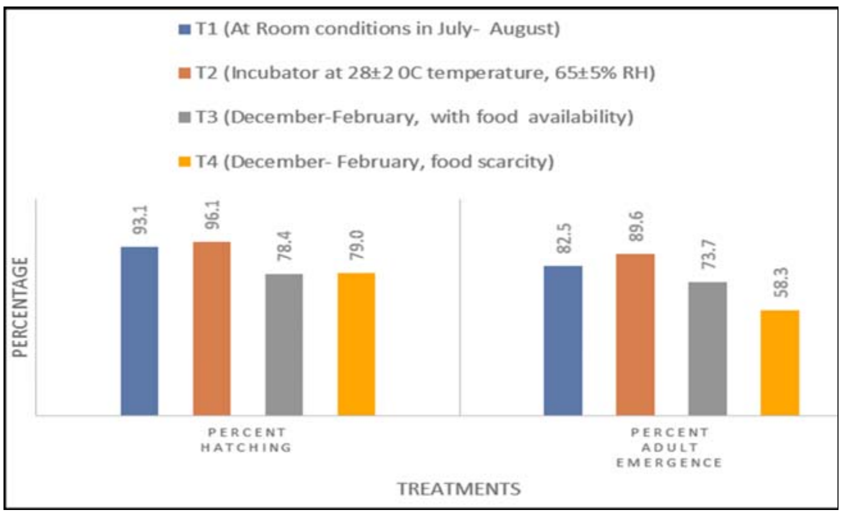

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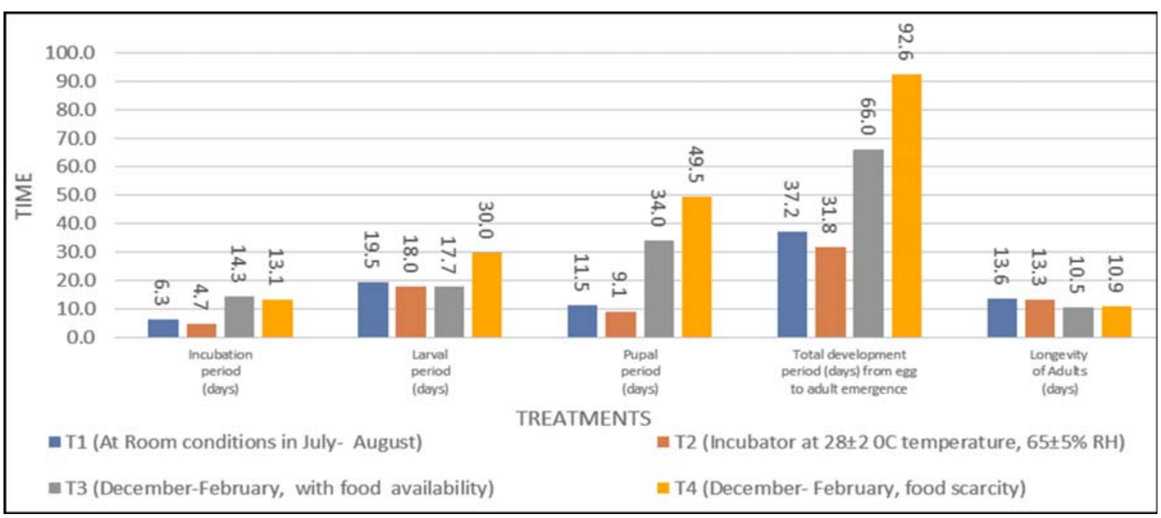

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Fig 2: Effect of temperature and humidity over the duration of Galleria mellonella ' s life cycle (Kumar and Khan, 2018) [51]. Graphs representing (a) fecundity, (b) hatching and adult emergence, (c) biology.

i) Temperature

Elevated temperatures lead to an increase in the inherent metabolic rate and energy demand in insects (Hiscox *et al*., 2016; Chen and Shakhnovich, 2010) [32, 18]. Proper housing conditions tailored to each stage of *G. mellonella* life cycle during rearing are crucial for their optimal growth. Containers housing the larvae should be ventilated as these larvae generate significant heat during feeding and development. Temperature significantly impacts the duration of *G. mellonella* 's life cycle. The larvae can thrive within a temperature range spanning from 20 °C to 42 °C. the temperature between 28 °C and 34 °C, the completion of its life cycle takes about 8-10 weeks (Table 1), while at room temperature (24 °C), this duration extends up to 13 weeks (Firacative *et al*., 2020) [24].

The developmental timeline of this pest varies notably, taking anywhere from 32 days to 93 days at temperatures of 28 °C and 2.5-24 °C, respectively (Kumar and Khan, 2018) [51]. Various reports by Desai *et al*. (2019) [21], Kumar and Khan (2018) [51], Swamy (2008) [90], and Pastagia and Patel (2007) [72] indicate that the pupal stage's duration ranges from 8 to 50 days based on temperatures ranging from 28 °C to 2.5-24 °C. Optimal larval development, as reported by Wojda *et al*. (2020) [99], Williams (1997) [97], and Warren and Huddleston (1962) [94], occurs within a temperature range of 29 °C to 33 °C. Remarkably, the larvae of this moth demonstrate resilience to diverse temperatures, including withstanding human body temperatures of 37 °C (Cook & McArthur, 2013) [19].

ii) Relative humidity

Maintaining controlled humidity levels within a ventilated environment is crucial to prevent contamination by fungal yeasts and molds, which could otherwise impede larval development (Pereira and Rossi, 2020; Buyukguzel and Buyukguzel, 2016) [74, 14]. Optimal growth necessitates a specific humidity level, as indicated by Pereira and Rossi (2020) [74] and Jindra and Sehnal (1990) [39]. Singh *et al*. (2016) [86] noted that lower humidity might extend the larval stage, while higher levels could expedite development, impacting pupation duration and adult emergence. Moreover, elevated humidity levels could lead to premature egg hatching. Both excessively high and low humidity can adversely affect pupal viability due to desiccation or mold growth (Jebabli *et al*., 2021) [36]. Studies, such as those by Moraes *et al*. (2020) [61], propose that humidity levels influence feeding rates and locomotor activity in *G. mellonella* larvae.

Hanumanthaswamy *et al*. (2013) [30] observed reduced adult longevity and fecundity at a low humidity range of 32-33%. At a relative humidity of 20%, Pereira and Rossi (2020) [74] and Chauvin and Chauvin (1985) [17] found a higher larval mortality rate. Negi *et al*. (2019) [63] reported that a relative humidity of 44-50% led to the highest incidence of *G. mellonella* in the *A. cerana* colony.

iii) Light

Light stands as a crucial abiotic factor influencing the larval development of *G. mellonella*. Continuous exposure to light leads to a decrease in insect size and hinders metamorphosis (Pereira and Rossi, 2020; Krrypsinl *et al*. 1974) [74, 48]. Smith *et al*. (2021) [88] delved into the disruptions caused by irregular light-dark cycles on *Galleria mellonella*, revealing disturbances in their physiological processes that impact stress responses and overall health. Alternating 12-hour light and dark cycles prompt early pupation (Pereira and Rossi, 2020; Bogus *et al*. 1987) [74, 9]. Dark environments foster increased mating and reproduction among these nocturnal insects (Joriao *et al*. 2018) [42], aligning with their nocturnal nature (Kwadha *et al*. 2017; Ellis *et al*. 2013) [52, 22]. Johnston *et al*. (2019) [40] explored the impact of distinct light wavelengths on *Galleria mellonella*, finding that some specific wavelengths affected larval behavior and immune responses. Specifically, blue light disrupted their natural behavior and feeding patterns, inducing alterations in growth and development. UV light exposure suppressed the larvae's immune systems, heightening susceptibility to infections.

Conclusion

The presence of *G. mellonella* starts in March, peaks in July, and gradually declines until the end of February. Both biotic and abiotic elements significantly influence *Galleria mellonella* abundance. In instances of starvation, cannibalism among early-stage larvae by their later-stage counterparts, alongside factors like nutritional content of food, and the presence of parasitoids such as *Bracon hebator* and *Dibrachys cayus*, as well as the bee species targeted, impact the abundance of *Galleria mellonella*. The ideal temperature range for normal development falls between 29-33℃, accompanied by a relative humidity range of 44-50%. Insect development in dark conditions leads to increased mating and reproduction.

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