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Suppressive Impact of 6-Benzyladenine, A Plant Growth Regulator, on the Adult Performance and Reproductive Potential of *Galleria mellonella* L. (Lepidoptera: Pyralidae)

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ABSTRACT

The greater wax moth *Galleria mellonella* is responsible for serious economic losses to bee keepers in developing countries. Larvae feed on the wax comb in weak colonies or during the storage of wax combs in winter. The objective of the current study was to assess the effect of the Cytokinin plant growth regulator, Benzyladenine (6-BA), on the most important parameters of adult performance and reproductive potential of *G. mellonella*. The 3rd instar larvae were force-fed on an artificial diet supplemented with six concentrations, *viz*., 100, 10.0, 1.0, 0.1, 0.01 & 0.001ppm of 6-BA. No adult moths could metamorphose at the higher three concentrations because of larval and pupal deaths. The most important results could be summarized as follows. The adult emergence was blocked by 6-BA, in a dose-dependent course. Also, the tested compound displayed strong adulticidal activity, since adult mortality increased in a dose-dependent course. The total adult longevity of moths was significantly prolonged.All longevity compartments were remarkably prolonged. On the other hand, 6-BA failed to affect adult morphogenesis. The oviposition efficiency was deleteriously prohibited. Also, 6-BA exhibited a tremendous inhibitory effect on the female fecundity and reducing effect onfertility. The embryonic development in eggs laid by treated adult females was severely retarded, since the incubation period of these eggs was remarkably prolonged.

INTRODUCTION

The greater wax moth *Galleria mellonella* L. (Lepidoptera: Pyralidae) is widely distributed throughout the world. It has been recorded in more than 77 countries (Kwadha *et al.*, 2017; Roh *et al.*, 2020). It is the major destructive pest for the apiculture industry because its larvae feed on the honeycomb, honey and wax found in bee hives, especially in weak colonies or during the storage of wax combs in winter (Büyükgüzel and Kalender, 2009), in addition to their tunneling habit through the combs (Chandel *et al.*, 2003).

Different physical and chemical methods have been used to control *G. mellonella* (Büyükgüzel, 2009). These methods and materials are very expensive and may have toxic effects on the bees. Furthermore, these methods may contaminate the bee products (Rortais *et al.*, 2017). In different parts of the world, the apiculture industry has traditionally relied on synthetic insecticides for controlling carious insect pests (Rehman *et al.*, 2009; Ilyas *et*

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al., 2017). These insecticides have dangerous impacts on non-target organisms and on the environment in general. They gradually accumulate in food materials and ultimately cause severe diseases in human consumers, like cancer, kidney failure and genetic disorders (Owain *et al.,* 2008; Ambethger, 2009; Łozowicka *et al.,* 2012; Mahdi and Mohammed, 2017). Also, one of the serious problems that emerged by the synthetic insecticide applications is the development of insect resistance toward the used insecticides (Yarahmadi *et al.,* 2009; Sparks and Nauen, 2015; Maazoun *et al.,* 2017). *G. mellonella* has developed high resistance to many insecticides (Said *et al.,* 2019).

Therefore, new environmentally safer alternative compounds should be searched (Ansari *et al.*, 2012; Glare *et al.*, 2016; Liao *et al.,* 2017; Kunbhar *et al.*, 2018). The use of natural products is a promising alternative for controlling *G. mellonella* because they generally have a low environmental contamination risk and there are few if any, harmful residues in the bee products (El-Wakeil, 2013; Farghaly *et al.*, 2017). Recently, the plant growth regulators (PGRs) have received increasing attention because they are environmentally friendly compounds of the plant origin (Abdellaoui *et al.*, 2015; Er and Keskin, 2016).Many authors (Kaur and Rup, 2002; Mendonça *et al.*, 2006; Gupta *et al.*, 2009; Tsagkarakis *et al.,* 2012; Prado and Frank, 2013; Kaur *et al*., 2016) reported that PGRs may have drastic effects on the survival, development and reproductive potential, as well as disturbance of other physiological processes and induction of the oxidative stress. Various authors (Altuntaş *et al.,* 2012; Uçkan *et al.,* 2014; Altuntaş, 2015), also, suggested that PGRs, such as gibberellic acid, ethephon and indole-3-acetic acid, could be used instead of synthetic insecticides to control some lepidopterous pests.

PGRs have been classified into different categories. Hopkins and Hüner (2004) classified the PGRs into six classes: Gibberellins (GAs), Auxins (Auxs), Ethylene (ET), Cytokinins (CTKs), Abscisic acid (ABA) and Brassinosteroids (BRs). Stamm *et al.* **(**2011) classified the PGRs into main nine classes: Auxs, GAs, CTKs, ET, ABA, BRs, salicylic acid (SA), Jasmonates (JAs) and Strigolactones (SLs). It is well known hitherto that CTKs have been produced in almost all higher plants as well as many mosses and prokaryotes (Salisbury and Ross, 1992). In addition, insects may produce CTKs, either directly or indirectly owing to their association with endosymbiotic bacteria (Giron and Glevarec, 2014; Zhang *et al.*, 2016). In insects, also, CTKs have been detected in saliva, labial glands, or/and accessory glands suggesting their ability to produce and deliver these compounds into plants as PGRs (Giron *et al*., 2013; Body et al., 2013; Bartlett and Connor, 2014). However, CTK has been reported to affect the morphology, development and behavior of some insects (Rup *et al.,* 1998).

In this context, the 6-Benzyladenine (6-BA) (or 6-Benzylaminopurine, BAP) is one of the most important synthetic CTKs. It is a plant growth stimulator with the chemical name: 4-hydroxyphenethyl alcohol. In plants, 6-BA can significantly increase CTK levels, of which levels are dramatically diminished under stress. Applying 6-BA was conducive to minimize adverse effects of environmental stress, such as drought, salt, low-temperature, and waterlogging stress (Majid *et al.*, 2011; Nurunnaher *et al*., 2014; Müller and Theron, 2018). For uses of 6-BA in agriculture, see Mangena (2020), Hu *et al.* (2020) and Bubán (2020). The objective of the current study was to assess the effect of 6-BA on the most important parameters of adult performance and reproductive potential of *G. mellonella*.

MATERIALS AND METHODS

The Insect:

A culture of asusceptible strain of the greater wax worm *Galleria mellonella* L. (Lepidoptera: Pyralidae) was established in the Department of Zoology, Faculty of Science, Al-Azhar University, Cairo, Egypt, and maintained for several successive generations under controlled conditions (27±2ºC, 65±5% R.H., photoperiod 14 h L and 10 h D). This culture was originally initiated by a sample of larvae kindly obtained from Desert Research Center, Cairo, Egypt. Larvae were transferred into glass containers, tightly covered with the muslin cloth. Different techniques for preparing the artificial diet had been described by some authors (Metwally *et al.*, 2012; Nitin et al., 2012). In the present culture of *G. mellonella*, an artificial diet was formulated depending on the method of Bhatnagar and Bareth (2004). The diet contained maize flour (400 g), wheat flour, wheat bran and milk powder, 200 g of each. Also, it was provided with glycerol (400g), bee honey (400g), yeast (100g). However, improved manipulation of different developmental stages had been done according to Ghoneim *et al.* (2019 a).

2. Plant Growth Regulator and Larval Treatment:

The compound 6-Benzyladenine (6-BA, or 6-Benzylaminopurine, BAP) is a plant growth stimulator (Cytokinin class) with the chemical name: 4-hydroxyphenethyl alcohol and molecular formula: $C_{12}H_{11}N_5$. It was purchased from Milipore Sigma, Burlington, MA 01803, USA Merk Ltd., Cairo, Egypt. A series of six concentration levels of 6-BA was prepared by diluting the compound with distilled water in volumetric flasks, as follows: 100.0, 10.0, 1.0, 0.1, 0.01 and 0.001 ppm.

Ten grams of the previously described artificial diet were supplemented with 2 ml of each concentration of 6-BA before introduction to the newly moulted 3rd instar larvae, as a food. These larvae were allowed to feed continuously on the treated diet throughout the larval stage. Control larvae were provided with distilled water-treated diet. Ten replicates of treated and control larvae (one larva/replicate) were kept separately in suitable glass vials under controlled laboratory conditions (27±2ºC, 65±5% R.H., photoperiod 14 h L and 10 h D). After force-feeding of 3rd instar larvae of diet supplemented with the higher three concentrations (100.0, 10.0 & 1.0ppm), larvae and pupae died. Therefore, all parameters of adult performance and reproductive potential were determined after feeding of similar larvae on diet mixed with the lower three concentrations of 6-BA (0.1, 0.01 & 0.001ppm).

Criteria of Study:

1. Adult Performance:

Adult Emergence: The number of the successfully emerged adults was expressed in %, according to Jimenez-Peydro *et al.* **(**1995**)**, as follows:

[No. of completely emerged adults / No. of pupae] × 100

Adult Mortality: It was calculated in % of the adult moth deaths.

Adult Morphogenesis: The impaired morphogenesis program of adult transformation was expressed in % of adult deformities.

Adult Longevity: The most important compartments of the longevity of adult females were measured in days (±SD): pre-oviposition period, oviposition period (reproductive lifetime) and post-oviposition period.

2. Reproductive Potential:

After feeding of 3rd instar larvae on an artificial diet (treated with 6-BA or control), the emerged adult moths were kept separately in glass jars (3 L) provided with white paper scraps, as oviposition sites. Each adult female was coupled with normal adult males (1:2) of the same age, obtained from the main culture. After mating, female moths were allowed to lay eggs. The eggs, singly or in patches, were collected daily, and carefully transferred into Petri dishes to be counted.

The Oviposition Efficiency: The oviposition efficiency was denoted by the **oviposition rate** which was calculated as follows:

Number of laid eggs per ♀/ reproductive lifetime (in days)

The Reproductive Capacity: The most important parameters of reproductive capacity were fecundity and fertility. **Fecundity:** the laid eggs were counted for calculating the number of eggs per female. **Fertility:** the hatchability was usually expressed in the hatching percentage of the laid eggs.**The sterility index** was calculated according to Toppozada*et al.*(1966) as follows:

$$Sterility\ Index = 100 – [(a\ b / A\ B) \times 100]$$

Where: a: mean number of eggs laid per female in the treatment. b: percentage of hatching in the treatment. A: mean number of eggs laid per female in the controls. B: percentage of hatching in the controls.

3. Incubation Period:

The laid eggs were kept in Petri dishes under the previously mentioned laboratory conditions. Just after the oviposition, eggs were observed until hatching to determine the incubation period (in days±SD).

4. Data Analysis:

Data obtained were statistically analyzed by the student's *t*-distribution, and refined by Bessel correction (Moroney,1956) for the test significance of the difference between means using GraphPadInStat© v. 3.01 (1998).

RESULTS

I. Effect of 6-Benzyladenineon the Adult Performance of *G. mellonella*:

After force-feeding of the 3rd instar larvae of *G. mellonella* on diet mixed with six concentrations of 6-Benzyladenine (6-BA**)** (100, 10.0, 1.0, 0.1, 0.01 & 0.001ppm), no adults could metamorphose at the higher three concentrations. Therefore, the adult performance parameters could be determined after larval feeding only on three sublethal concentrations. Data of these parameters were summarized in Table (1).

1.1. Effect of 6-BA on the Adult Emergence:

In the light of data assorted in the previously mentioned table, the adult emergence was blocked, in a dose-dependent course (20, 60 & 80% emergence of treated adults, at 0.1, 0.01 & 0.001ppm, respectively, compared to 100% emergence of control adults).

1.2. Effect of 6-BA on Adult Survival:

Depending on the same table, 6-BA displayed strong adulticidal activity, since adult mortality increased by the increasing concentration (80, 40 & 20% mortality of treated adults, at 0.1, 0.01 & 0.001ppm, respectively, compared to 0% mortality of control adults).

1.3. Effect of 6-BA on Adult Morphogenesis:

No deformed adults could be observed, i.e., the 6-BA failed to affect the adult morphogenesis.

1.4. Effect of 6-BA on Adult Longevity:

As obviously shown in Table (1), the total adult longevity was significantly prolonged, in a dose-dependent course (17.2±0.28, 16.4±0.30 & 14.2±0.34 days of treated adults, at 0.1, 0.01 & 0.001ppm, respectively, *vs*. 13.2±0.30 days of control adults). One of the major time compartments of adult longevity is the pre-oviposition period (may be theovarian maturation period). This period was generally prolonged. Moreover, it was pronouncedly prolonged at the highest concentration (12.1±0.42, 3.0±0.14 & 3.4±0.42 days of treated adults, at 0.1, 0.01 & 0.001ppm, respectively, *vs*. 3.2±0.35 days of control adults).

Another major time compartment of adult longevity is the oviposition period (reproductive lifetime). Also, this period was considerably prolonged (7.0±0.42, 6.1±0.14 & 5.7±0.70 days of treated adults, at 0.1, 0.01 & 0.001ppm, respectively, *vs*. 5.5±0.56 days of control adults). In addition, the last time compartment is the post-oviposition period. It was remarkably prolonged (7.2±0.14, 6.5±0.14 & 6.1±0.70 days of treated adults, at 0.1, 0.01 & 0.001ppm, respectively, *vs*. 4.6±0.28 days of control adults, Table 1).

Table1: Effect of 6-Benzyladenine on the adult performance parameters of *G. mellonella* after force-feeding of 3rd instar larvae on treated artificial diet.

Parameter / Conc. (ppm)	Adult emergence (%)	Adult mortality (%)	Adult longevity (mean days±SD)			
			Pre-oviposition period (Ovarian maturation period)	Oviposition period (Reproductive life-time)	Post-oviposition period	Total Longevity
0.1	20	80	12.1±0.42 c	7.0±0.42 c	7.2±0.14 d	17.2±0.28 d
0.01	60	40	3.0±0.14 a	6.1±0.14 a	6.5±0.14 d	16.4±0.30 d
0.001	80	20	3.4±0.42 a	5.7±0.70 a	6.1±0.70 c	14.2±0.34 c
Control	100	00	3.2±0.35	5.5±0.56	4.6±0.28	13.2±0.30

Conc.: concentration. Mean±SD followed with (a): insignificantly different (P>0.05). (c): highly significantly different (P<0.01),(d): very highly significantly different (P<0.001).

2. Effect of 6-BA on the Reproductive Potential:

After force-feeding of 3rd instar larvae of *G. mellonella* on an artificial diet supplemented with six concentrations of 6-BA (100, 10.0, 1.0, 0.1, 0.01 & 0.001ppm), successfully emerged and reproduced adult female moths were observed only at the three lower concentrations. Data of the most important criteria of the reproductive potential were summarized in **Table (2).**

2.1. Effect of 6-BA on Oviposition Efficiency:

According to data of this table, the oviposition efficiency was deleteriously prohibited by 6-BA, since the oviposition rate was drastically regressed, in a dose-dependent course (26.6±0.28, 33.4±0.56 & 42.0±1.41 of treated adult females, at 0.1, 0.01 & 0.001ppm, respectively, *vs*., 45.8±0.56 of control adult females).

2.2. Effect of 6-BA on Reproductive Capacity:

Data of the same table revealed a tremendous inhibitory effect of 6-BA on the female fecundity (mean number of eggs/♀), in a dose-dependent manner (80.8±2.82, 121.3±1.41 & 189.5±1.41 eggs /treated♀, at 0.1, 0.01 & 0.001ppm, respectively, compared to 229.9±2.82 eggs /control♀, Table 2).

Another informative parameter of the reproductive capacity is fertility (hatching% of laid eggs or egg viability) which was significantly reduced, (63.9±0.14, 69.5±0.70 & 70.1±0.14 hatching eggs laid by treated ♀♀, at 0.1, 0.01 & 0.001ppm, respectively, compared to 70.3±0.42 hatching eggs laid by control ♀♀). It may be important to estimate the sterilityindex which increased parallel to the increasing concentration of 6-BA (69.69, 48.21 & 18.49, at 0.1, 0.01 & 0.001ppm, respectively, Table 2).

2.3. Effect of 6-BA on Embryonic Development:

In insects, the incubation period of eggs can be used as an informative indicator of the embryonic developmental rate, i.e., a longer period usually denotes as lower rate of embryogenesis and *vice versa*. On the basis of data assorted in Table (2), the embryonic development in eggs laid by treated adult females was severely retarded with a noticeably slower rate, since the incubation period of these eggs was significantly prolonged (9.3±0.42, 9.0±0.70 & 8.9±0.14 days of eggs laid by treated adult females, at 0.1, 0.01 & 0.001ppm, respectively, compared to 7.4±0.28 days of eggs laid by control adult females).

Table 2: Effects of 6-Benzyladenine on the oviposition and reproductive capacity of *G. mellonella*, after force-feeding of 3rd instar larvae on treated artificial diet**.**

Conc. (ppm)	Oviposition rate (mean±SD)	Reproductive capacity			Incubation period (Mean days ±SD)
		Fecundity (mean no. of eggs ±SD)	Fertility (mean %±SD)	Sterility index (%)	
0.1	26.6±0.28 d	80.8±2.82 d	63.9±0.14 d	69.69	9.3±0.42 d
0.01	33.4±0.56 d	121.3±1.41 d	69.5±0.70 b	48.21	9.0±0.70 c
0.001	42.0±1.41 c	189.5±1.41 d	70.1±0.14 a	18.49	8.9±0.14 d
Control	45.8±0.56	229.9±2.82	70.3±0.42	----	7.4±0.28

Conc., a, c, d: see footnote of Table (1). (b): significantly different ($P<0.05$).

DISCUSSION

I. Disruption of Adult Performance of *G. mellonella* by 6-Benzyladenine:

I.1. Blocked Adult Emergence:

It is important to emphasize that the adult emergence in insects is a crucial physiological process and regulated by some hormones, particularly the eclosion hormone. Disturbance of this hormone partially or completely arrests the adults to emerge **(**Josephrajkumar *et al.*, 1999; Ghoneim *et al.*, 2019 b).

In the present study, force-feeding of 3rd instar larvae of *G. mellonella* on diet supplemented with three sublethal concentrations of 6-Benzyladenine (6-BA) led to the blockage of adult emergence, in a dose-dependent course. The present result was in agreement with many reported results of blocked adult emergence after larval treatment of various insects with some plant growth regulators (PGRs), such as the melon fruit fly *Bactrocera cucurbitae* after feeding of larvae on artificial diets containing different concentrations of gibberellic acid (GA_3)(Kaur and Rup, 1999) or Coumarin (Cn), kinetin, GA_3 and indole-3-acetic acid (IAA) (Kaur and Rup, 2003); the tobacco cutworm *Spodoptera litura* by rearing the newly hatched larvae on artificial diets fortified with miraculan (Singh and Bhattacharya, 2001; Bhatnagar *et al.*, 2012) or GA_3 (Shiwani and Karnatak, 2012) and the mustard aphid *Lipaphis erysimi* after treatment of nymphs with GA_3, Daminozide(Alar-B9), indole-3-butyric acid (IBA) or Chlorogenic acid (Rup *et al.*, 2002). On the contrary, our result disagreed with very few reported results of enhanced adult emergence of some insects after larval treatments with some PGRs, such as the Egyptian cotton leafworm *Spodoptera littoralis* after feeding of larvae on diet supplemented with GA_3 (Salama and El-Sharaby, 1972) and *G. mellonella* after injection of Abscisic acid (ABA) into the haemocoel (Er and Keskin, 2016).

For interpretation of the blocked adult emergence of *G. mellonella* after force-feeding of 3rd instar larvae on diet mixed with 6-BA, in the currentstudy, this compound might interfere with the eclosion hormone release and/or inhibition of the neurosecretion (prothoracicotropic hormone) (Al-Sharook *et al.*, 1991; Josephrajkumar *et al.*, 1999). The 6-BA might exhibit a disturbing effect on the normal metabolism of insect hormones during the development of the immatures leading to failure of adult emergence (Trigo *et al.*, 1988). On the molecular basis, 6-BA might cause misexpression of certain genes, particularly the brood complex (*br-C*) transcription factor gene, leading to symptoms of impaired metamorphosis, like blocking of adult emergence (Wilson, 2004; Nandi and Chakravarty, 2011).

I.2. Adulticidal Activity of 6-BA:

After force-feeding of 3rd instar larvae of *G. mellonella* on diet supplemented with 6-BA, in the current investigation, the compound displayed strong adulticidal activity, since adult mortality increased by the increasing concentration. In the available literature, no information was found on the chronic toxicity of PGRs against adults of insects after treatment of larvae. Apart from PGRs, different plant-derived compounds were reported to exhibit adulticidal activities against some insects, such as Thymoquinone against the maize weevil *Sitophilus zeamais* (Herrera *et al.*, 2015); *trans*-cinnamaldehyde, (−)-menthone and eugenol against the rice weevil *Sitophilus oryzae* (Saad *et al.*, 2018); Nerolidol (Hamadah *etal.*, 2020) and Farnesol (Hamadah *et al*., 2021) against *S. littoralis*.

To interpret the chronic toxic effect of 6-BA on adult moths of *G. mellonella*, in the present study, this PGR might be retained and distributed in the body, as a result of direct and rapid transport from the gut of treated larvae into other tissues, through haemolymph, to the adults and by lower detoxification capacity of adults against the tested PGR (Osman *et al.*, 1984; Smagghe and Degheele, 1992). Because the adult life in insects depends on healthy immature stages, the digestive disorders may be the cause of untimely adult mortality, as recorded for 6-BA against *G. mellonella* adults in the current study (Soltani, 1984).Also, an extended toxic effect of 6-BA might be due to the disturbance of enzymatic pattern and/or hormonal hierarchy in adults of *G. mellonella*(Kartal*et al.*, 2003).In addition, the adult mortality of *G. mellonella* might be explicated by a latent inhibitory effect of 6-BAon the feeding leading to continuous starvation and subsequently death (Ghoneim et al., 2000) or adverse effect on the homeostasis leading to increased loss of the body water and subsequently death (Amer *et al.*, 2004).

I.3. Disturbed Adult Longevity by 6-BA:

I.3.1. Total Adult Longevity:

There are many reported results of shortened total adult longevity of different insects after treatment with various PGRs. For example, thetotal adult longevity of *G. mellonella* moths was shortened after feeding of larvae on diet treated with GA_3 (Uckan *etal.*, 2011) or mebendazole (Calık *et al.*, 2016) or by injection of ABA into the haemocoel (Er and Keskin, 2016). Also, feeding of *S. littoralis* larvae on a diet supplemented with GA_3 resulted in shortened total adult longevity (Salama and El- Sharaby, 1972). Exposure of the migratory grasshopper *Melanopliss anguinipes* nymphs to Ethylene led toshortened female adult longevity (Chrominski *et al.*, 1982). The total adult longevity of *B. cucurbitae* was shortened by GA_3 treatments (Kaur and Rup, 2002). The total adult longevity of wolfberry aphid (Gong *et al.*, 2010) or brown planthopper *Nilapar vatalugens* (Senthil-Nathan *et al.*, 2009) was shortened after treatment with Jasmonic acid (JA). In a recent study by Nagaratna *et al.* (2021), feeding of larvae of the fall armyworm *Spodoptera frugiperda* on maize treated with silicic acid (FSA), GA_3 and JA, separately or in combination, resulted in thes hortening of total adult male longevity by all treatments but total adult female longevity was unaffected.

The present results disagreed with the previously reported results since force-feeding of 3rd instar larvae of *G. mellonella* on diet supplemented with 6-BA resulted in significantly prolonged total adult longevity, in a dose-dependent course. However, the current literature contains no information about the prolongation of total adult longevity of insects after treatment with PGRs except a recent study by Çelik and Sak (2021) who investigated the effects of kinetin against *A. grisella* and recoded significantly prolonged adult longevity of males, at 5 mg/Lkinetin.

Interpretation of the shortened total adult longevityof different insects after larval treatments with various PGRs is available (Abdel-Aal, 1996; Broughton *et al.,* 2005; Carbone *et al.,* 2006; Kefford *et al.,* 2008; Chamseddin *et al.,* 2012; Er *et al.,* 2017) while the exact interpretation of prolongation of total adult longevityof *G. mellonella* after larval feeding on diet mixed with 6-BA, in the present study, is unfortunately available right now. On the other hand, insects often show degenerative changes in some tissues and organs, after the attainment of sexual maturity, which can be called 'senility' or 'aging'. The affected adult longevity can be considered as an informative indicator for adult aging, i.e., prolongation of longevity may denote a delay of aging but shortened longevity may denote an acceleration of aging, although the death is usually the density of all creatures (for review see Ghoneim and Bakr, 2018). Depending on our results, 6-BA seemed to delay the aging of *G. mellonella*, i.e., it exhibited antioxidant activity against this insect. However, the exact mode of action of 6-BA on the biochemical sites in adults is unknown until now.

I.3.2. Prolonged Pre-Oviposition Period:

To the best of our knowledge, no information is available on the effects of PGRs on major compartments of the adult longevity (pre-oviposition period, oviposition period and post-oviposition period) of insects, except one study conducted by Abdellaoui *et al.* (2009) on *L. migratoria* who topically applied GA_3 or injected it into oesophagus of the newly emerged adults and recorded a prolongation of the pre-oviposition period. Apart from PGRs, Nerolidol (Hamadah *et al.,* 2020) and Farnesol (Hamadah *et al.,* 2021) inhibited the adult female moths of *S. littoralis* to pass generally prolonged pre-oviposition period. Results of the present study were, to some extent, in agreement with those reported results, since force-feeding of 3rd instar larvae of *G. mellonella* on a diet mixed with 6-BA led to a general prolongation of the pre-oviposition period of successfully mated adult female moths.

Many lepidopterous species have a relatively short adult stage, or even non-feeding adults, like *G. mellonella*. In these insects, anadult female emerges with most of her eggs ready to be fertilized within a day or very few days. The prolonged pre-oviposition period of *G. mellonella*in the present study may be informative to delaying or retarding the effect of 6-BA on the finishing steps of ovarian maturation (Ghoneim and Al-keridis, 2019**)**. In addition, ovarian maturation in insects is known to be under endocrine control (Kaur and Rup, 2002). The delayed ovarian maturation in *G. mellonella* by 6-BA, in the current work, seemed to be related to its interference with the inhibition of ecdysteroid production in the ovaries, the occurrence of which is a prerequisite for the developing ovaries (Acheuk et al., 2012). However, the exact mode of delaying effect of 6-BA on the ovarian maturation of *G. mellonella*, in the current investigation, is unfortunately not available right now. On the other hand, the interference of this compound with the hormonal regulation of this physiological process needs further investigation in the foreseeable future.

I.3.3. Prolonged Oviposition Period:

Another major time compartment of adult longevity is the oviposition period (reproductive lifetime). Depending on the currently available literature, there are no studies examining the effects of PGRs on this period other than the study of Abdellaoui *et al.* (2009) who topically applied or injected different concentrations of GA_3 into the oesophagus of the newly emerged adult females on *L. migratoria* and recorded a reduction of the oviposition period. Aside from PGRs and *G. mellonella*, treatment of *S. littoralis* larvae with Nerolidol (Hamadah *et al.,* 2020) or Farnesol (Hamadah *et al.,* 2021) resulted in a remarkably shortened oviposition period of the successfully mated female moths.

Results of the present study clearly disagreed with those reported results, since force-feeding of 3rd instar larvae of *G. mellonella* on a diet mixed with 6-BA led to considerable prolongation of the oviposition period of adult females. The shortened oviposition period can be understood as a result of enforcing the effect of the tested compound on adult females to quickly lay eggs during a very short interval to avoid this toxic xenobiotic factor (Tanani and Ghoneim, 2018) while the prolongation of this period after treatment of *G. mellonella* larvae with 6-BA, in the present study, could not be explicated right now!!

I.3.4. Prolonged Post-Oviposition Period:

The last time compartment of the adult longevity in insects is the post-oviposition period. From the current literature, no reliable information has been obtained regarding the effects of PGRs, or other plant-derived compounds, on this time interval of adult longevity in insects. Only Ghoneim *et al.* (2019 b) recorded a considerably shortened post-oviposition period of the successfully mated adult females of *G. mellonella* after treatment of 3rd instar larvae with the honey bee venom,Apitoxin. Our result is contradictory to this reported result since the post-oviposition period of *G. mellonella* was considerably prolonged after larval treatment with 6-BA. Unfortunately, there is no acceptable interpretation for this prolongation right now!!

I.4. Deranged Adult Morphogenesis by 6-BA:

In the current study, 6-BA failed to affect the adult morphogenesis, since no adult deformities of *G. mellonella* had been observed after force-feeding of 3rd instar larvae on a diet mixed with different concentrations of this PGR. In contrast to our result, very few studies examined the effects of PGRs on morphogenesis of some insects. Yeşilada *et al.* (1996) observed adult abnormalities of the vinegar fly *Drosophila melanogaster* after treatment of different developmental stages with kinetin and ABA. Kaur and Rup (2003) observed some morphological deformities in adult flies of *B. cucurbitae* after treatment of larvae with Cn, GA_3, kinetin, or IAA. Recently, Çelik and Sak (2021) investigated the effects of kinetin against *A. grisella* and observed moths with curved wings, shortened wings.

Apart from PGRs, different plant-derived compounds had been reported to adversely affect the morphogenesis of some insects. For example, treatment of the confused flour beetle *Tribolium confusum* larvae with Andrographolide resulted in the production of some deformed adults (Lingampally *et al.*, 2013). Feeding of 2nd instar larvae of*S. litura* on fresh food treated with Allyl isothiocyanate resulted in the metamorphosis of some deformed adults (Bhushan *et al.*, 2016). Larval treatment of *S. litura* and *Spodoptera exigua* withPogostone resulted in some deformities of the adult moths (Huang *et al.*, 2014). Topical application of Farnesol onto 5th instar nymphs of the red cotton stainer *Dysdercus koenigii* led to the metamorphosis of adults with malformed wings (Kumar and Gupta, 2017). Sosa *et al.* (2019) observed serious wing malformations in adults of *S. frugiperda* after larval treatments with some Sesquiterpene lactone compounds. Feeding of *S. littoralis* 2nd instar larvae on plant leaves previously dipped in Nano-chitosansolution led to the production of deformed adult moths (Marouf, 2020). Different malformations of *S. littoralis* adults had been observed after larval treatments with the sesquiterpene compounds, Nerolidol (Hamadah *et al.*, 2020) and Farnesol (Hamadah *et al.*, 2021).

II. Impaired Reproductive Potential of *G. mellonella* by 6-BA:

Great research attention has been focused on the drastic activities of PGRs against the reproduction of insect pests. For example, a reduction in the reproductive rate of some species of aphids was reported after feeding on some plants treated with chlormequat chloride (Chch) (Honeyborne, 1969). Mepiquat chloride (Mch) reduced the reproductive rate of wheat aphid *Schizaphis graminum* (Dreyer *et al.*, 1984). A similar result of impaired reproduction was obtained for the same aphid species on GA_3-treated sorghum crops (Campbell *et al.*, 1984). Also, Cytokinin (CTK) has been reported to affect the reproduction of mustard aphid *Lipaphis erysimi* (Rup *et al.*, 2000). In addition to aphids, GA_3 reduced the reproductive potential of the Mediterranean fruit fly *Ceratitis capitata* (Barbouche and Ben Hamouda, 1986; Hussein, 2005).

II.1. Prohibited Oviposition Efficiency by 6-BA:

In the present study on *G. mellonella*, the oviposition efficiency of adult females was deleteriously prohibited, since the oviposition rate was drastically regressed, in a dose-dependent course, after force-feeding of 3rd instar larvae on diets mixed with different concentrations of 6-BA. The present result was in corroboration with the reported results of prohibited oviposition of some insects by certain PGRs, such as the Asian citrus psyllid *Psylla citri* by PDC, Uniconazole or Mefluidide (Tsagkarakis *et al.,* 2012). Also, other studies showed that kinetin reduced the egg-laying capacity of the fruit flies (Kaur and Rup, 2002). Apart from PGRs, various plant-derived compounds had been reported to detrimentally inhibit the oviposition efficiency of some insect pests, such as *S. littoralis* by Nerolidol (Hamadah *et al.*, 2020) or Farnesol (Hamadah *et al.*, 2021); *S. oryzae* by Eugenol (Chaubey, 2016); the melon worm *Diaphania hyalinata* by Rotenone (Silva *et al.*, 2016) and the vinegar fly *Drosophila suzukii*by citral and eugenol (Eben *et al.*, 2020).

To interpret the inhibited oviposition efficiency of *G. mellonella* after force-feeding of the 3rd instar larvae on diet mixed with 6-BA, in the present study, it is important to point out that the reproduction in insects is mainly controlled by juvenile hormone (known as 'gonadotropic hormone' in adults). This hormone is responsible for the protein metabolism for egg maturation (Ghoneim *et al.*, 2014). On the other hand, ecdysteroids play a crucial role in the control of different processes involved in female reproduction, such as vitellogenesis and ovulation of matured eggs (Wigglesworth, 1984; Hagedorn, 1985). In the light of this information, the inhibited oviposition efficiency, in the current study, might be due to the interference of 6-BA with these hormones during the vitellogenesis *via* certain biochemical processes or might exert a reverse action to those exhibited by ecdysteroids which induce the neurosecretory cells to release a myotropic ovulation hormone (Smagghe *et al.*, 1996; Parween *et al.*, 2001). In addition, eggs might develop normally in ovaries of the adult females of *G. mellonella*, but they could not be lay, owing to the adversely deformed ovipositor by the action of 6-BA, or to the reduced mechanical strength (Moreno et al., 1994) or their reabsorption before oviposition (Zhou *et al.*, 2016).

II.2. Deteriorated Reproductive Capacity of *G. mellonella* by 6-BA:

II.2.1. Prohibited Fecundity*:*

After force-feeding of 3rd instar larvae of *G. mellonella* on diet mixed with 6-BA, the present study revealed a tremendous inhibitory effect of this PGR on the adult female fecundity (mean number of eggs/♀), in a dose-dependent manner. This result was in agreement with many reported results of inhibited fecundity of different insect species after treatment with various PGRs. For example, an early study was conducted by Robinson **(**1960) in which he recorded fecundity reduction of the pea aphid *Acyrthosiphon pisum* after feeding on broad bean treated withmaleic hydrazide (MH). Some years later, the 3rd instar nymphs ofthe same aphid species were fed on diets containing 0.5- 2.0% MH for three days leading to significantly reduced fecundity (Bhalla and Robinson, 1968). The fecundity ofthe black bean aphid *Aphis fabae* was inhibited when 1-15 ppm GA_3 was sprayed on *Vicia fabae* (Honeyborne, 1969). Chlormequat chloride (Chch) had been found to reduce the fecundity of several aphid species (Honeyborne, 1969; van Emden, 1969). After treatment ofnymphs of the cotton stainer bug *Dysdercus cardinalis* with 2000 ppm chlormequat chloride (Chch), the emerged adult females had less fecundity (Carlisle et al., 1969). Feeding of *S. littoralis* larvae on a diet supplemented with GA_3 (Salama and El-Sharaby, 1972) or Weedazol (3-amino-1,2,4 triazol 25%) (Dimetry and Mansour, 1976) led to severe reduction of the adult fecundity. After addingGA_3 and ABA to a grass diet of the bigheaded grasshopper *Aulocaraelliotti*, significantly reduced fecundity was determined by Visscher (1980). The exogenous JA treatment of the wolfberry plant resulted in significantly reduced adult fecundity of the wolfberry aphid *Aphis* sp., in a dose-dependent course (Gong *et al.*, 2010).

The lemon trees *Citrus volkameriana* were treated with PDC, Uniconazole, or Mefluidide. The rearing of Asian citrus psyllid *D. citri* on the treated trees led to significant reduction in adult fecundity (Tsagkarakis *et al.,* 2012). The injection of ABA into the haemocoel of *G. mellonella* resulted in a reduction of fecundity, at higher doses (Er and Keskin, 2016). Feeding of *G. mellonella* larvae on Mebendazole-treated diet from 1st instar larvae to the last instar resulted in significantly reduced fecundity of the producing female moths (Calıket al., 2016). Application of GA_3 (at 0.5 mg plant^{-1}) on maize, as a host plant, significantly reduced the fecundityof *S. frugiperda* (Nagaratna *et al.,* 2021).

On the other hand, our result disagreed with few reported results of increasing fecundity of some insects after treatment with certain PGRs, such as *A. elliotti* after feeding on adiet treated with 10 and 20 mg/L doses of kinetin (Visscher, 1982); *D. melanogaster* after feeding on diet mixed with high doses (10^{-3} and 10^{-4}M) of kinetin (Yeşilada and Bozcuk, 1996); the Silverleaf whitefly *Bemisiatabaci* after feeding on tomato seedlings sprayed with IAA (Di *et al.,* 2014) and the mulberry silkworm *Bombyx mori* after topical application of three doses of each GA_3 and Kinetin (Sepperumal and Sukumar, 2014).

To explicate the drastically inhibited adult fecundity of *G. mellonella*, after feeding of 3rd instar larvae on diet mixed with 6-BA, in the current study, this PGR might interfere with one or more physiological processes, from the ovarian follicle development to egg maturation. However, some suggestions could explain the presently prohibited fecundity, as follows. (1) 6-BA might cause some disorders in the ovaries, including cell death in the germarium, resorption of oocytes in the pre-vitellarium and vitellarium, formation of vitellin envelops and undue proliferation of follicle cells sometimes resulting in malformation of the whole ovary (Lucantoni *et al.,* 2006; Khan *et al.,* 2007). (2) The 6-BA might inhibit the development of some ovarioles and/or the synthesis and metabolism of proteinaceous constituents during the oogenesis (Salem *et al.,* 1997). (3) The 6-BA might exert an inhibitory action on the ecdysone activity, the threshold of which is crucial for normal oogenesis (Terashima *et al.,* 2005).(4) On the basis of hormonal regulation of insect reproduction, 6-BAmight disturb the production and/or function of the gonadotropic hormone (JH in adults) responsible for the synthesis of vitellogenins (yolk precursors) and vitellogenesis (Di Ilio*et al.,* 1999).(5) It may be acceptable to suggest that the prohibited fecundity may be due to theinhibitory effect of 6-BA on a synthesis of both DNA and RNA, suboptimal nutrition owing to reduced feeding, altered mating behaviour as a result of sublethal intoxication, or a combination of factors.

II.2.2. Reduced Fertility*:*

Another informative parameter of the reproductive capacity is fertility (hatchingpercentage of laid eggs or egg viability) which was remarkably reduced after force-feeding of 3rd instar larvae of *G. mellonella* on diet mixed with 6-BA, in the present study. Also, the sterility index increased parallel to the increasing concentration of 6-BA. This result was in accordance with many reported results of the fertility reduction in several insects after treatment with various PGRs. For example, feeding of the tobacco budworm (*Chloridea virescens*) larvae on a diet supplemented with GA_3 resulted in areduction of the hatching percentage of eggs laid by the successfully emerged adult females (Guerra, 1970). A similar result was recorded for *S. littoralis* by the same PGR (Salama and El-Sharaby, 1972). El-Ibrashy *et al.* (1976) injected Alar®85 into the newly moulted gregarious 4th instar nymphs of the desert locust *Schistocerca gregaria* and recorded complete sterility (zero fertility) in the treated locust. Adding GA_3 or ABA to a grass diet of *A. elliotti* significantly reduced the egg viability (Visscher, 1980). Treatment of the speckled rangeland grasshopper *Arphia conspersa* with a concentration of 60 mg/1 of ABA led to reduced fertility (Jurenka, 1982). Topical application of para-Aminobenzoic acid (vitamin B_x) onto *B. mori* larvae caused a significant reduction in the egg hatchability (Pai *et al.,* 1986). JA was reported to reduce the egg hatchability in the brown planthopper *Nilapar vatalugens* (Senthil-Nathan *et al.,* 2009). Feeding of *G. mellonella* larvae on a mebendazole-treated diet from 1st instar larvae to the last instar resulted in significantly reduced hatchability of the laid eggs by adult females (Calık *et al.,* 2016). Application of GA_3 (at 0.5 mg plant^{-1}) on maize, as a host plant, significantly reduced the fertilityof *S. frugiperda* (Nagaratna et al., 2021). Recently, Çelik and Sak (2021) investigated the effects of kinetin against the smaller wax moth *Achroia grisella*. They found that kinetin, failed to affect egg fertility. On the contrary, our result disagreed with some exceptional cases of enhanced fertility of some insects by certain PGRs, such as the increasing fertility of the grasshopper *Aulocara elliotti* females after feeding on a diet mixed with 10 and 20 mg/L doses of kinetin (Visscher, 1982).

The detrimentally reduced fertility of *G. mellonella* adult females after force-feeding of 3rd instar larvae on diet supplemented with 6-BA could be understood in the light of the following suggestions. (1) Maturation of the insect eggs depends basically on the vitellogenins, precursor materials of vitellins including proteins, lipids and carbohydrates, all of which are necessarily required for embryonic development (Soltani and Mazouni, 1992; Chapman, 1998). These materials are synthesized primarily by the fat body during the immature stages (Telfer, 2009) or by the ovary *in situ* (Indrasith et al., 1988). The 6-BAmight disturb the production of vitellogenins and/or accumulation in adult females, leading to the reduction of fertility. (2) The 6-BA might indirectly affect fertility *via* its disruptive effect on the opening of the intracellular spaces in follicular epithelium or generally inhibited the role of the gonadotropic hormone responsible for the regulation of vitellogenin deposition into oocytes (Davey and Gordon, 1996; Büyükgüzel, 2006). (3) The reduction in fertility might be due to the penetration of residual amounts of 6-BA in the adult females into their eggs and disturbance of embryonic cuticle synthesis. So, the fully mature embryos had weakened chitinous mouth parts that were insufficiently rigid to perforate the surrounding vitellin membrane and free from the eggs (Sallam, 1999; Sammour et al., 2008). (4) The reduced fertility might be due to the serious effect of 6-BA on survival of the developing embryos at certain stages, as recorded in decreasing hatching percentage.

II.2.3. Retarded Embryonic Development by 6-BA:

In insects, the incubation period of eggs can be used as an informative indicator of the embryonic developmental rate, i.e., a longer period usually denotes a slower rate of embryogenesis and *vice versa*. In the present study, 3rd instar larvae of *G. mellonella* were force-fed on diet mixed with 6-BA. The embryonic development in eggs laid by adult female moths was severely retarded with a slow rate since the incubation period of these eggs was significantly prolonged. To our knowledge, there was no reliable information in the available literature regarding the effects of PGRs on the embryonic development or incubation period of eggs, except a study by Uçkan *et al.* (2011). Our result was in corroboration with their result of the prolonged incubation period of eggs laid by *G. mellonella* after rearing larvae on diet treated with >1.0 ppm of the GA_3. On the other hand, the currently available literature contains very few studies examining the effect of some plant-derived compounds of the incubation period, such as the retarded embryonic development, or prolonged incubation period of eggs, after treatment of *S. littoralis* larvae with the Sesquiterpene compounds, Nerolidol (Hamadah *et al.,* 2020) and Farnesol (Hamadah *et al.*, 2021). The retarded embryonic development in *G. mellonella*, in the present study, might be due to an impairing effect of 6-BA on the ecdysteroids responsible for the regulation of certain stages of embryogenesis, especially those ecdysteroids originating from the ovary *in situ* (Chapman, 1998).

Conclusion:

Depending on the present results, the tested Cytokinin compound,6-Benzyladenine (6-BA) exhibited a considerable blocking effect on the adult emergence of *G. mellonella*, high insecticidal activity against adult moths, in addition to its prohibitory effect on oviposition efficiency, drastically reducing effect on fecundity and fertility, as well as it retarded the embryonic development in the laid eggs. Therefore, 6-BA may be an effective PGR being used in the IPM program against this serious pest of apiculture.

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