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**Toxicity and Bio-Efficacy of Indole-3-Acetic Acid, A Plant Growth Regulator,
Against the Greater Wax Moth, *Galleria mellonella* Linnaeus
(Lepidoptera: Pyralidae)**

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ABSTRACT

The greater wax moth *Galleria mellonella* L. is a dangerous pest for the apiculture industry in the world. The objective of the present study was to evaluate the insecticidal activity of the auxin plant growth regulator, Indole-3-acetic acid (IAA), against *G. mellonella* and its effect on the growth, development, metamorphosis and morphogenesis of this insect pest. 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 & 0.001 ppm of IAA. The most important results could be summarized as follows. IAA exhibited the strongest acute toxicity on larvae at the higher two concentrations. At other concentrations, various degrees of toxicity on larvae were found in a dose-dependent course. No pupae or adults were produced at the higher two concentrations. IAA failed to display chronic toxicity on the developed pupae. All emerged adults died at 1.00 ppm of IAA but no adult mortality was observed at the three lower concentrations. LC₅₀ value was calculated in 0.24 ppm. IAA enhanced the treated larvae to grow with increasing weight gain. Also, it promoted the treated larvae, since the growth rate increased with the increasing concentration. IAA exhibited a retarding effect on the pupation, only at the higher two concentrations. The larval and pupal durations were significantly prolonged, in a dose-dependent manner. On the other hand, IAA failed to disturb the hormonal regulation of the development program, since no intermediate creatures were produced. Also, it failed to disrupt the pupal morphogenesis, since no pupal deformities had been observed.

INTRODUCTION

The greater wax moth *Galleria mellonella* Linnaeus (Pyralidae: Lepidoptera) is a cosmopolitan serious insect for the apiculture industry (Kwadhaet *al.*, 2017). It can be found in beehives or stored waxes because its larvae feed on the honeycomb, honey and

wax (Büyükgüzel and Kalender, 2009; Roh *et al.*, 2020). It is responsible for serious economic losses to beekeepers in developing countries (Nurullahoglu and Susurluk, 2001; Viraktamath, 2010). Although the adults of *G. mellonella* do not feed, because they have atrophied or ill-developed mouth parts, the voracious feeding nature of its larvae leads to the destruction of the honeycomb, and subsequent to the death of weak colonies (Ellis *et al.*, 2013; Elbehery *et al.*, 2016). Furthermore, it can destroy wax combs either inside or outside the hives (Awasthi and Sharma, 2013; Kwadha *et al.*, 2017). Besides damaging wax combs and destroying frames and wooden parts in the hive, adults and larvae of *G. mellonella* can transfer pathogens of serious bee diseases, e.g. the bacterial disease foulbrood (Charrière and Imdorf, 1997; Owayss and Abd-Elgayed, 2007).

In the last few decades, several techniques have been developed to control *G. mellonella*. Different mechanical and chemical methods have been applied outside the hives (Ellis *et al.*, 2013). On the other hand, the control measures inside the hives are very limited (Abou-Shaara, 2017). In addition, the physical, chemical and biological methods are insufficient (Ali *et al.*, 1974; Burges, 1981). However, some chemicals had been reported for controlling *G. mellonella* by some manipulations in the hive and other treatments to stored combs (Calderone, 2000; Owayss and Abd-Elgayed, 2007). Although the use of these chemicals is somewhat easy and effective, some precautions of safety and contamination of bee products are considered. Moreover, some of these chemicals seemed to be ineffective against eggs of *G. mellonella* (Owayss and Abd-Elgayed, 2007). Although synthetic pesticides are capable of killing a wide range of agricultural pests, their overuses are responsible for many environmental problems (Czeher *et al.*, 2008, Yadouleton *et al.*, 2010) in addition to their hazardous effects on human health (Whitehorn *et al.*, 2012; Hallmann *et al.*, 2014). To avoid the previously mentioned hazards, it is important to search for new effective and safe alternatives with negligible effects on the ecosystem (Dubey *et al.*, 2010; Chandler *et al.*, 2011; Korrat *et al.*, 2012). One promising alternative may be the use of plant growth regulators (PGRs) against pest species. Several researchers have focused on the effects of various PGRs on herbivores, since these compounds may have considerable impacts on the survival, development and reproductive potential, as well as on other physiological processes and induction of the oxidative stress of many insect pests (Tsagkarakis *et al.*, 2012; Prado and Frank, 2013; Abdellaoui *et al.*, 2015; Kaur *et al.*, 2016). Also, many authors (Kaur and Rup, 2002; Silva *et al.*, 2003; Paulson *et al.*, 2005; Abdellaoui *et al.*, 2013) have even suggested the use of certain PGRs, like gibberellic acid (GA₃) and Indole-3-acetic acid (IAA), as successful chemosterilants against insect pests.

Over the last few years, several entomologists have been investigating the PGRs and their adverse effects on the biology and physiology of some insects, such as the greater wax moth *G. mellonella* (Altuntaş *et al.*, 2012; Er and Keskin, 2016). To the best of our knowledge, the two major classes: gibberellins and auxins, among different PGRs, had been widely tested against *G. mellonella* (Uçkan *et al.*, 2014, 2015; Altuntaş *et al.*, 2012, 2014). Auxins (Auxs) were discovered as the first class of PGRs (Zhao, 2010). Aux is produced at the shoot apex in young leaves and actively moves down but not upwards into buds (Ljunget *et al.*, 2001). To the best of our knowledge, one of the most interesting Aux compounds is IAA, a plant growth stimulator with the chemical name: 2-(1H-indol-3-yl) ethanoic acid. Because of the wide usage of the indolic compounds as PGRs in the environment, non-target organisms, such as biological control agents could be negatively affected, many authors (Rup *et al.*, 2002; Kaur and Rup, 2003; Uçkan *et al.*, 2011; Uçkan *et al.*, 2014; Uçkan *et al.*, 2015; Çelik *et al.*, 2017) reported that IAA caused adverse effects on survival, developmental duration, adult longevity, reproductive potential, hemocytes responses and haemolymph metabolites of various lepidopterous pest species. However,

very few studies thoroughly examined the insecticidal, biological, physiological and/or haemotological activities of PGRs against *G. mellonella*. Therefore, the objective of the present study was to evaluate the insecticidal activity of IAA against *G. mellonella*, and its effect on the growth, development, metamorphosis and morphogenesis of this insect pest.

MATERIALS AND METHODS

1. Culturing of the Greater Wax Moth:

A culture of a susceptible 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 ($27\pm 2^\circ\text{C}$, $65\pm 5\%$ 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 a 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, b).

2. Plant Growth Regulator and Larval Treatment:

The compound Indole-3-Acetic Acid (IAA) is a plant growth stimulator. It is a synthetic auxin compound with the chemical name: 2-(1H-indol-3-yl) ethanoic acid and molecular formula: $\text{C}_{10}\text{H}_9\text{NO}_2$. It was purchased from Milipore Sigma, Burlington, MA 01803, USA Merk Ltd., Cairo, Egypt. A series of six concentration levels of IAA 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 mixed with 2 ml of each concentration of IAA before introduction to the newly moulted 3rd instar larvae, as a food. These larvae were allowed to continuously feed 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 ($27\pm 2^\circ\text{C}$, $65\pm 5\%$ R.H., photoperiod 14 h L and 10 h D). All survival and biological criteria were recorded daily after the first 24 hrs of feeding.

3. Criteria of Study:

3.1. Insecticidal Activity:

All mortalities of treated and control larvae, pupae and adults were recorded daily. The total mortality was corrected according to Abbott's formula (Abbott, 1925) as follows

$$\% \text{ Corrected mortality} = \frac{\% \text{ test mortality} - \% \text{ control mortality}}{100 - \% \text{ control mortality}} \times 100$$

LC₅₀ values were calculated for general mortality by Microsoft® office Excel (2007), according to Finny (1971).

3.2. Growth, Development and Metamorphosis:

Larval Body Weight Gain: Each individual larva (treated or control) was carefully weighed daily, using a digital balance, for calculating the body weight gain as follows:

Initial weight (before the beginning of experiment) - final weight (at the end of experiment)

The growth rate was calculated according to Waldbauer (1968) as follows:

Fresh weight gain during feeding period / feeding period x means fresh body weight of larvae during the feeding period.

Developmental duration and rate: Dempster's equation (1957) was applied for calculating the developmental duration. Richard's equation (1957) was used for calculating the developmental rate.

Metamorphosis: Pupation rate was expressed in % of the successfully developed pupae. The **deranged metamorphosis programs** were detected and calculated in larval-pupal or pupal-adult intermediates (%). **Pupal water loss** was calculated depending on the data of the initial and final weights of the pupae, as follows:

$$\text{Water loss \%} = [\text{initial weight} - \text{final weight} / \text{initial Weight}] \times 100$$

Morphogenesis: The impaired morphogenesis program of pupation was expressed in % of pupal deformities.

4 Statistical Analysis of Data:

Data obtained were 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

1. Toxic Effect of IAA on Different Developmental Stages of *G. mellonella*:

After force-feeding of 3rd instar larvae of *G. mellonella* on diet mixed with six concentrations of IAA (100, 10.0, 1.0, 0.1, 0.01 and 0.001 ppm), data of its insecticidal activity were assorted in Table (1). Depending on these data, IAA exhibited the strongest acute toxicity on larvae at the two higher concentrations, since all treated larvae died, compared to 0% mortality of control larvae. At other concentrations, IAA exhibited various degrees of toxicity in a dose-dependent course (80, 40 & 20% larval mortalities, at 1.0, 0.1 & 0.01 pp, respectively, vs. 0% mortality of control larvae. Moreover, no mortality was recorded for IAA at its lowest concentration. In the light of these data, no pupae or adults were produced at 100 & 10.0 ppm IAA. The present PGR failed to display chronic toxicity on the successfully developed pupae because no pupal mortality was observed. Depending on data of the same table, force-feeding of larvae on 1.00ppm IAA-treated diet resulted in complete mortality of the successfully emerged adults (100% adult mortality, at 1.0 ppm, vs. 0% mortality of control congeners) while no adult mortality had been recorded at the three lower concentrations. However, the corrected mortality was determined in a dose-dependent manner, with an exception of the lowest concentration, at which IAA failed to affect the survival of *G. mellonella*. LC₅₀ value was calculated at 0.24 ppm (Fig. 1).

Table 1: Toxicity of Indole-3-acetic acid against *G. mellonella* (as expressed in mortality%) after force-feeding of 3rd instar larvae on treated artificial diet.

Develop. stage Conc. (ppm)	Larvae	Pupae	Adults	Total	Corrected	LC ₅₀ (ppm)
100.0	100	---	---	100	100	0.24
10.0	100	---	---	100	100	
1.0	80	0	100	100	100	
0.1	40	0	00	40	40	
0.01	20	0	00	20	20	
0.001	00	0	00	00	00	
Control	0	0	0	0	---	

Conc.: Concentration level, Develop.: Developmental.

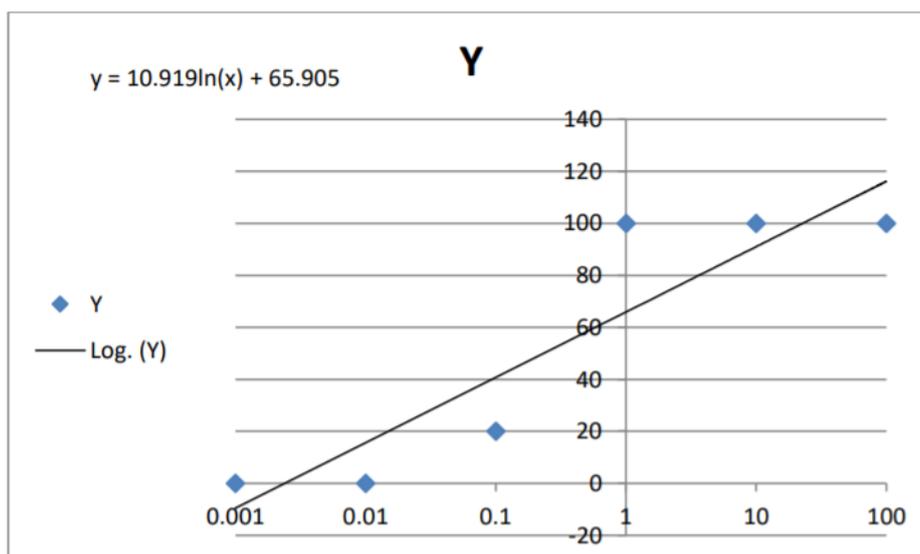


Fig. 1: Regression line to calculate LC_{50} of Indole-3-acetic acid against *G. mellonella* after force-feeding of 3rd instar larvae on treated artificial diet.

2. Effect of IAA on Growth, Development, Metamorphosis And Morphogenesis of *G. mellonella*:

After continuous force-feeding of the 3rd instar larvae of *G. mellonella* on diet supplemented with the sublethal concentrations of IAA, data of the affected growth and development were arranged in Table (2).

2.1. Affected Somatic Weight Gain and Growth Rate:

As clearly shown in Table (2), somatic weight gain of the treated larvae considerably increased, in a dose-dependent course (80.0 ± 0.70 , 61.0 ± 1.41 , 60.2 ± 0.28 & 60.0 ± 0.14 mg, at 1.0, 0.1, 0.01 & 0.001 ppm, respectively, compared to 55.8 ± 1.13 mg of control larvae). Thus, IAA enhanced the treated larvae to grow with increasing weight. Also, this PGR promoted the growth of treated larvae, since the growth rate increased with the increasing concentration (193.2 ± 0.28 , 117.2 ± 0.28 , 110.6 ± 0.84 & 108.0 ± 1.41 , at 1.0, 0.1, 0.01 & 0.001 ppm, respectively, vs. 78.9 ± 1.27 of control larvae, Table 2).

2.2. Perturbed Developmental Durations and Rate:

On the basis of data contained in the previously mentioned table, the duration of the IAA-treated larvae was highly significantly prolonged, in a dose-dependent manner (29.2 ± 0.28 , 28.7 ± 0.98 , 25.5 ± 0.42 & 25.5 ± 0.70 days, at 1.0, 0.1, 0.01 & 0.001 ppm, respectively, vs. 23.4 ± 0.56 days of the control larvae). In addition, the pupal duration was remarkably prolonged, almost in a dose-dependent course (10.2 ± 0.28 , 10.1 ± 1.14 , 10.0 ± 2.82 & 8.40 ± 0.56 days, at 1.0, 0.1, 0.01 & 0.001 ppm, respectively, vs. 8.20 ± 0.28 days of the control pupae). Data of Table (2), also revealed that the developmental rate was slightly regressed in no certain trend (4.27, 4.60, 4.48 & 4.44, at 1.0, 0.1, 0.01 & 0.001 ppm, respectively, compared to 4.71 of control congeners).

2.3. Disrupted Developmental and Metamorphosis Programs:

Depending on data assorted in Table (2), IAA treatment led to a weak retarding effect on the pupation process, since 80 & 80% of pupation were determined at the higher two concentrations but no effect was exhibited by the compound at other concentrations. Also, the water loss might indicate the deaths of pupae, since increasing water loss% was determined in a parallel trend to the IAA concentration (51.4, 39.8, 30.1 & 29.6, at 1.0, 0.1, 0.01 & 0.001 ppm, respectively, in comparison with 28.7% water loss of control pupae, Table 2).

Table 2: Effect of Indole-3-acetic acid on growth and development of *G. mellonella* after force-feeding of 3rd instar larvae on treated artificial diet

Develop. Stage Conc. (ppm)	Larval stage				Pupal stage		
	Weight gain (mean mg±SD)	Growth rate (mean ±SD)	Duration (mean days ±SD)	Develop. rate	Pupation (%)	Duration (mean days ±SD)	Water loss (%)
1.0	80.0±0.70 d	193.2±0.28 d	29.2±0.28 d	4.27	80.0	10.2±0.28 d	51.4
0.1	61.0±1.41 d	117.2±0.28 d	28.7±0.98 d	4.60	80.0	10.1±1.14 b	39.8
0.01	60.2±0.28 d	110.6±0.84 d	25.5±0.42 d	4.48	100	10.0±2.82 d	30.1
0.001	60.0±0.14 d	108.0±1.41 d	25.5±0.70 d	4.44	100	8.40±0.56 a	29.6
Control	55.8±1.13	78.9±1.27	23.4±0.56	4.71	100	8.20±0.28	28.7

Conc., Develop.: see footnote of Table (1). Develop. stage: Developmental stage. Mean±SD followed with (a): insignificantly different ($P>0.05$). (b): significantly different ($P<0.05$), (d): very highly significantly different ($P<0.001$).

It was strikingly that IAA failed to adversely affect the development program (larval-pupal transformation), since no indicating features, like the larval failure of ecdysis or the production of larva-pupal intermediates had been observed. Moreover, the tested PGR failed to disturb the hormonal regulation of development, since no extra moult, supernumerary larval instar, superlarvae, or permanent larvae, had been observed. Also, this PGR failed to impair the pupation program, since no pupal deformities had been recorded. Furthermore, IAA had no anti-morphogenic property on *G. mellonella*.

DISCUSSION

Affected Survival of *G. mellonella* by Indole-3-acetic Acid (IAA):

Various plant growth regulators (PGRs) had been reported to exhibit insecticidal activities against *G. mellonella*, such as Abscisic acid (ABA) which caused mortality of last instar larvae in a dose-dependent dose (Er and Keskin, 2016); Ethephon (ETF) exhibited considerable acute toxicity to *G. mellonella* larvae (Altuntaş *et al.*, 2016); rearing of *G. mellonella* larvae on mebendazole-treated diet resulted in some mortalities of last instar larvae (Calık *et al.*, 2016).

Results of the present study were, to some extent, in accordance with the previously reported results, since IAA exhibited the strongest acute toxicity on *G. mellonella* larvae at the higher two concentrations and various degrees of toxicity at other concentrations. IAA failed to display chronic toxicity on the developed pupae or emerged adults except for its 1.00ppm at which the emerged adults completely died. Also, the present results may be in corroboration with some reported results of different PGRs' toxicities against various insects, such as miraculan and milstim (Harikesh and Blattacharya, 2001) and Gibberellic acid (GA_3) (Shiwani and Karnatak, 2012) against the tobacco cutworm *Spodoptera litura*; GA_3 against the Egyptian cotton leafworm *Spodoptera littoralis* larvae and the migratory locust *Locusta migratoria* nymphs (Abdellaoui *et al.*, 2009, 2013); Coumarins (Cns) against the melon fly *Bactrocera cucurbitae* (Kaur and Rup, 2003) and the fall armyworm *Spodoptera frugiperda* (Vera *et al.*, 2006); GA_3 and jasmonic acid (JA) against *S. frugiperda* (Nagaratna *et al.*, 2021); Siapton against the jute hairy caterpillar *Spilarctia oblique* (Gupta *et al.*, 2009); and kinetin against the lesser wax moth *Achroia grisella* (Çelik and Sak, 2021).

It may be important to explicate the toxicity of IAA, against larvae of *G. mellonella* in the current investigation. Some scenarios could be suggested as follows. (1)

IAA may possess an insecticidal property *per se* or by conversion into toxic molecules after ingestion (Lajide *et al.*, 1993). (2) The hormone ecdysone plays a major role in shedding old cuticles in a process known as "moulting" in insects. When IAA entered into larvae, the ecdysone activity was inhibited and subsequently, the larvae failed to moult, and ultimately died (Jeyasankar *et al.*, 2013; Sivaraman *et al.*, 2014; Chennaiyan *et al.*, 2016). In addition, IAA might interfere with the synthesis or/and deposition of chitin on the criticized internal structures, such as the peritrophic matrix, leading to death (Merzendorf and Zimoch, 2003; Merzendorf, 2005). (3) It is possible that the insecticidal property of IAA might disrupt some metabolic activities in larvae resulting in death (Jeyasankar *et al.*, 2014; Chennaiyan *et al.*, 2016). (4) The larval deaths might be due to the prevention of feeding and continuous starvation of the treated larvae (Ghoneim *et al.*, 2000). To interpret the toxic effect of IAA 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, and by lower detoxification capacity of adults against the tested PGR (Osman *et al.*, 1984).

In respect of LC₅₀ of IAA against *G. mellonella*, in the present study, it was calculated at 0.24 ppm. Other PGRs had been reported with various LC₅₀ values against some insects since ETF exhibited LD₅₀ for force ETF-fed larvae as 344 µg/5 µl (Altuntaş *et al.*, 2016) and Cytokinin (CTK) exhibited LC₅₀ (380 ppm) against mustard aphid *Lipaphis erysimi* (Rup *et al.*, 2000). Apart from PGRs, Er *et al.* (2017) topically applied Azadirachtin onto *G. mellonella* larvae and determined LC₅₀ in 16.564 ppm. The Sesquiterpene compound epi-β-bisabolol showed high toxicity against early 3rd instar larvae of the malaria mosquito *Anopheles stephensi* with LC₅₀= 14.68 µg/ml, the yellow fever mosquito *Aedes aegypti* with LC₅₀= 15.83 µg/ml and the southern house mosquito *Culex quinquefasciatus* with LC₅₀= 17.27 µg/ml (AlShebly *et al.*, 2017). Another sesquiterpene compound, Farnesol, exhibited toxicity against *Aphis craccivora* and *Leucania separata* with LC₅₀ values of 20.2 and 15.2 mg L⁻¹, respectively (Tang *et al.*, 2011). Also, Ghoneim *et al.* (2020) determined LC₅₀ values of Farnesol after treatment of penultimate instar larvae and last instar larvae of *S. littoralis* as 36.56 and 33.67 ppm, respectively. However, LC₅₀ values usually depend on several factors, such as susceptibility of the insect and its treated stage or instar, lethal potency of the tested compound or product and its concentration, method and time of treatment or exposure, as well as the experimental conditions (Ghoneim *et al.*, 2017).

Disrupted Growth and Development of *G. mellonella* by IAA:

Neumann (1982) reported that the supplementation of food with different PGRs had been demonstrated to deleteriously affect the growth, development and other physiological processes in several insects, such as on *B. cucurbitae* (Kaur and Rup, 2002), *S. oblique* (Gupta *et al.*, 2009), *G. mellonella* (Altuntaş *et al.*, 2012; Er and Keskin, 2016) and the solitary endoparasitoid *Apanteles galleriae* (Uçkan *et al.*, 2014).

1. Affected the Larval Weight and Growth:

PGRs had been reported to diversely affect the larval body weight and growth in various insects, since some PGRs enhanced the larval body weight and growth rate, while other PGRs reduced the body weight and inhibited the larval growth. Early, El-Ibrashy and Mansour (1970) demonstrated that chlormequat chloride (Chch) reduced the larval growth of black cutworm *Agrotis ypsilon* on castor bean leaves. Feeding of the corn earworm *Helicoverpa zea* larvae on Mepiquat chloride (Mch)-treated cotton plants resulted in the reduction of larval body weight (Zummo *et al.*, 1984). Four PGRs, *viz.*, Cn, kinetin, GA₃ and IAA, had been applied onto *B. cucurbitae* larvae. All compounds exerted growth-inhibitory effects on larvae (Kaur and Rup, 2003). JA reduced the body weight of final instar larvae of *S. frugiperda* after feeding on treated rice (Singh and Bhattacharya, 2003),

cotton (Meszaros *et al.*, 2011), or soybean (Gordy *et al.*, 2015). Feeding of *S. littoralis* larvae and *L. migratoria* nymphs on diet treated with GA₃ resulted in significantly reduced larval body weight in both insects (Abdellaoui *et al.*, 2009). Rearing the newly hatched larvae of *S. litura* on an artificial diet supplemented with GA₃ resulted in a gradual decrease of growth with the increasing concentration (Shiwani and Karnatak, 2012). Feeding of *S. litura* larvae on an artificial diet mixed with Miraculan resulted in suppression of larval growth, in a dose-dependent course (Singh and Bhattacharya, 2001; Bhatnagar *et al.*, 2012). Recently, the foliar treatment of maize with JA against *S. frugiperda* resulted in reduced larval weight (Nagaratna *et al.*, 2021).

Results of the present study on *G. mellonella* disagreed with the previously reported results, since the somatic weight gain of the IAA-treated larvae considerably increased, in a dose-dependent course. Also, IAA promoted the growth of treated larvae, since the growth rate was found increasingly with the increasing concentration. Our results were in agreement with several reported results of increasing larval body weight and/or enhanced growth rate of different insects by various PGRs. For example, feeding of the bollworm *Heliothis virescens* larvae on diet supplemented with GA₃ resulted in increasing larval body weight (Guerra, 1970). The larvae of the tobacco hornworm *Manduca sexta* grew significantly larger on plants treated with 10 µM GA₃, compared to the control plants (Fabisch, 2013). Tanjung (2007) recorded increasing body weight of the last instar (5th) larvae of the mulberry silkworm *Bombyx mori* after treatment with GA₃. Raghunath and Narayanaswamy (2013) topically treated the 5th instar larvae of *B. mori* with GA₃ and observed the highest growth rate of larvae at 100 µg/ml. Also, topical application of GA₃ onto *B. mori* caterpillars led to the significantly increased larval weight of the 5th instar larvae (Sepperumal and Sukumar, 2014).

In this context, explication of the reduction of larval body weight in insects by PGR treatments may be provided by some authors (Neumann, 1982; Bhattacharya *et al.*, 2011). On the contrary, the increased larval body weight gain and promoted growth rate of *G. mellonella* after treatment with IAA, in the current study, might be due to a phagostimulatory action of this PGR on the larval appetite, nutrition and absorption of plant material (Visscher, 1982).

2. Prolonged Developmental Durations And Regressed Developmental Rate:

Literature sources indicate that some PGRs failed to affect the development rate and developmental durations of some insects. For example, neither Cch nor Mch had any effect on the larval duration of *S. oblique*, regardless of the dose added to the artificial diet (Singh and Bhattacharya, 2002). Also, the application of GA₃ had no influence on the pupal duration of *S. oblique* (Gupta *et al.*, 2009). In a recent study, the foliar treatments of maize with GA₃ and JA against *S. frugiperda* resulted in no significant effect on larval duration (Nagaratna *et al.*, 2021). Çelik and Sak (2021) investigated the effect of kinetin against *A. grisella* and found no significant change in the development duration.

In contrast, force-feeding of 3rd instar larvae of *G. mellonella* on diet mixed with different concentrations of IAA, in the present study, resulted in significantly prolonged larval and pupal durations, in a dose-dependent manner. Also, the developmental rate was significantly regressed. These results were consistent with many reported results of prolongation of the developmental durations and regressed developmental rate of different insects by various PGRs, such as significantly prolonged larval duration of the bivoltine *B. mori* after topical application with IAA (Hugar and Kaliwal, 1997); a prolongation of the larval and pupal durations of the banana fruit fly *Zaprionus parvittiger* was reported when their diet contained low doses of kinetin (25 ppm and above) (Rup *et al.*, 1998); a prolongation of the developmental period of *B. cucurbitae* was recorded after larval feeding on an artificial diet containing GA₃ (Kaur and Rup, 1999) or Cn, kinetin or IAA (Kaur and

Rup, 2003); GA₃ prolonged the larval period of *S. oblique* at the recommended and higher doses (Gupta *et al.*, 2009); feeding of *S. litura* larvae on an artificial diet fortified with Miraculan resulted in prolonged larval and pupal durations (Bhatnagar *et al.*, 2012); injection of ABA into the haemocoel of the *G. mellonella* larvae led to prolongation of larval duration (Er and Keskin, 2015).

On the other hand, the present results disagreed with the reported results of shortened developmental durations and promoted developmental rate, such as significantly shortened larval duration after topical application of GA₃ or Kinetin (Sepperumal and Sukumar, 2014) or 2,4-Dichlorophenoxy acetic acid (2,4-D) and Naphthoxy acetic acid (NOA)(Goudar and Kaliwal, 2001) onto caterpillars of *B. mori*; Cch caused a significant shortening in the pupal period in *S. oblique*, at certain concentrations (Gupta *et al.*, 2009) and shortened developmental duration of *G. mellonella* after feeding the 1st instar larvae to the last instar on Mebendazole -treated diet (Calik *et al.*, 2016).

To explicate the prolongation of larval and pupal duration and retarded developmental rate of *G. mellonella* after force-feeding of 3rd instar larvae on diet mixed with IAA, in the current study, the present PGR might indirectly interfere with the neuroendocrine organs responsible for the synthesis and release of tropic hormones, such as prothoracicotrophic hormone (Subrahmanyam *et al.*, 1989), or interfere with some endocrinal metabolic processes involved in development (Kaur and Rup, 2002). The prolongation of larval duration might be due to decreased food intake, caused by phagodeterrence of IAA (Awad and Ghazawy, 2016), or by a deviation of part of the taken food to the detoxification metabolism (Tanzubil and McCaffery, 1990). With decreased food ingestion and low biomass conversion, the insect takes longer to reach the critical weight for ecdysis, leading to the prolongation of larval duration (Giongo *et al.*, 2015). Also, the final step of the chitin biosynthesis pathway might be inhibited by IAA and the precursor was not converted into chitin leading to a prolongation of the developmental period (Djehader *et al.*, 2014).

3. Inhibited Pupation:

In the present study, force-feeding of 3rd instar larvae of *G. mellonella* on diet mixed with IAA led to suppression of the pupation rate (as expressed in decreased pupation%) only at the higher two concentrations. This result was, to a great extent, in accordance with some reported results of reduced pupation of some insects after treatment with various PGRs. For example, the pupation rate in *B. cucurbitae* was reduced when the larvae were fed an artificial diet containing GA₃ (Kaur and Rup, 1999). To a great extent, a similar reduction of pupation was recorded in *S. litura* after larval feeding on a Miraculan-treated diet (Bhatnagar *et al.*, 2012) and in *G. mellonella* after injection of ABA into the larval haemocoel (Er and Keskin, 2015).

Apart from PGRs, different plant products or plant-derived compounds hindered the pupation process of various insect species (Jilani *et al.*, 2006; Kaur *et al.*, 2010; Kaur *et al.*, 2017; Gupta *et al.*, 2017), such as *S. frugiperda* after treatment with eucalyptin, chrysin, eucalyptin, quercetin, luteolin, and betulinic and oleanolic acids (Salazar *et al.*, 2015); *S. litura* after feeding of 3rd instar larvae on diet mixed with alantolactone and isoalantolactone (Kaur *et al.*, 2017) and *S. littoralis* after larval treatment with Farnesol (Ghoneim *et al.*, 2020) or Nerolidol (Ghoneim *et al.*, 2021).

To understand the hindered pupation or regressed pupation rate of *G. mellonella*, in the current investigation, IAA might exhibit an inhibitory effect on the synthesis of specific storage proteins in the fat body during the last larval instar and their deposition at the time of pupation (Gupta, 1985). IAA might exert a suppressive action on the chitin synthesis and prevented the normal deposition of the new cuticle during apolysis (Retnakaran *et al.*, 1985). IAA might disrupt the ecdysteroid metabolism or might

alternatively act directly to inhibit the release of an ecdysis-triggering hormone (Gaur and Kumar, 2010).

Disturbed Metamorphosis and Morphogenesis Programs of *G. mellonella* by IAA:

In insects, the disturbing development or metamorphosis program can be indicated by failure of ecdysis of larvae or the production of extra moult, superlarva, permanent larvae, larval-pupal, or/and pupal-adult intermediates. All or some of these symptoms or features had been recorded for certain insects by some plant-derived compounds (Kaur *et al.*, 2014; Palanikumar *et al.*, 2017), such as the confused flour beetle *Tribolium confusum* after treatment with Andrographolide (Lingampally *et al.*, 2013), *S. litura* after treatment with Andrographolide (Edwin *et al.*, 2016), *S. littoralis* after treatment with farnesol (Ghoneim *et al.*, 2020) or nerolidol (Ghoneim *et al.*, 2021). As far as our literature survey could ascertain, no information was available on the impairing effects of PGRs on the development or metamorphosis program. On the other hand, only scarce studies examined the disruptive effects of certain PGRs on the morphogenesis program of insects. To our knowledge, the only study involved in this context was conducted by Abdellaoui *et al.* (2009). They observed impaired external morphology of *L. migratoria migratoria*, since different nymphal deformities had been produced after feeding on a GA₃-treated diet. In addition, the same authors reported some disorders in the internal morphology of the same locust. In the present study on *G. mellonella*, IAA failed to adversely affect the metamorphosis program (larval-pupal transformation), since no indicating features, like larval failure of ecdysis or the production of larva-pupal intermediates had been observed. Moreover, the tested PGR failed to disturb the hormonal regulation of development, since no extra moult, supernumerary larval instar, superlarvae, or permanent larvae, had been observed. In addition, the tested IAA failed to impair the morphogenesis program, since no pupal deformities had been recorded.

CONCLUSION:

Depending on results in the present study, Indole-3-acetic acid (IAA) exhibited insecticidal activity against larvae and adults of *G. mellonella*, at higher concentrations, while it failed to affect the survival of pupae. It enhanced larvae to grow with an increasing growth rate. It caused significant prolongation of larval and pupal durations. Moreover, it exhibited a weak retarding effect on the pupation process and failed to adversely affect the hormonal regulation of metamorphosis and morphogenesis programs. Therefore, IAA could not be recommended as an effective control agent against *G. mellonella*.

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