

Evaluation of Regulatory Influence of Four Plant Growth Regulators on the Reproductive Potential and Longevity of Melon Fruit Fly (*Bactrocera cucurbitae*)

Rabinder Kaur and P.J. Rup^{*,1}

The topical treatment given to freshly emerged (0–1-day-old) male and female adults of *Bactrocera cucurbitae* (Coquillett), a serious pest of cucurbit crops in tropical countries, with 25, 125, 625 and 3125 ppm concentrations of gibberellic acid (GA₃), indole-3-acetic acid (IAA), kinetin and coumarin showed a significant adverse influence on the reproductive potential of this fruit fly. The assessment for reproductive potential was made on the basis of reduction in fecundity and fertility of laid eggs and measured as sterility in females and shortening of the longevity, *i.e.* ovipositional phase. The strongest influence was with kinetin, followed closely by coumarin, then GA₃ and lastly with IAA treatments. It was concluded that although these compounds demonstrate their activities differently in plants and might be following a different mode of action in insects, they ultimately influence the reproductive potential of this insect.

KEY WORDS: *Bactrocera cucurbitae*; gibberellic acid; indole-3-acetic acid; kinetin; coumarin; reproduction; longevity.

INTRODUCTION

The growth regulatory compounds of the plants, directly or indirectly, are bound to play a vital role in the patterns of growth and reproduction of associated phytophagous insects. This presumption receives support from reports in the literature demonstrating their influence on these parameters, when added to the diet or applied exogenously on the insects, in the case of abscisic acid (ABA), gibberellic acid (GA₃), kinetin, etc. (1,13,23,26). Some workers have even recommended the use of plant growth regulators (PGRs) like GA₃, coumarin, IAA, etc., as successful chemosterilants against some insect pests (12,19,24). In the present day search for eco-friendly compounds to replace the traditional synthetic organopesticides, a thorough and specific investigation of these compounds might yield some promising candidate compounds for use in the IPM programme of economically important insect pests.

The fruit flies of the family Tephritidae (Diptera), such as *Anastrepha suspensa* (Loew), *Ceratitis capitata* (Wiedemann), *Bactrocera cucurbitae* (Coquillett) and *Dacus dorsalis* Hendel, are serious pests of many fruit and vegetable crops. They oviposit eggs directly on fruits and their larvae feed on the pulp, destroying the crop's maturing fruit. In the literature, some workers have reported adverse effects of GA₃ and IAA on the growth

Received July 27, 2001; received in final form Nov. 24, 2001; <http://www.phytoparasitica.org> posting March 18, 2002.

¹Insect Physiology Laboratory, Dept. of Zoology, Guru Nanak Dev University, Amritsar 143 005, India. *Author for correspondence [e-mail: pushp@angelfire.com].

and reproduction of *A. suspensa*, *C. capitata* and *D. dorsalis* (4,5,10,24,25). The melon fly *B. cucurbitae* is the commonest and most destructive pest of cucurbit fruits in tropical countries including India, sometimes causing total loss of yield, and to date has defied the conventional control measures with organopesticides. The PGRs like GA₃, IAA and kinetin have been observed to affect adversely the growth and development of this fruit fly (7,8,14). The present investigation is an attempt to explore the effects of four PGRs, *i.e.*, GA₃, IAA, kinetin and coumarin (belonging to four main classes of phytohormones, or mimicking their activities), on the longevity and reproductive potential of this fruit fly.

MATERIALS AND METHODS

The effects of GA₃, IAA, kinetin and coumarin on the reproductive potential and longevity of the melon fruit fly, *B. cucurbitae*, were investigated by exposing the freshly emerged adult flies (0–1 day old) to four different concentrations (25, 125, 625 and 3125 ppm) of these PGRs. The compounds were purchased from Loba Chemie Private Ltd., Mumbai (India). The solutions of GA₃, IAA, kinetin and coumarin were prepared in ethanol, ethanol 0.1N NaOH and hot distilled water, respectively. The colony flies were reared in stock by adopting the procedure described by Gupta and co-workers (6). The flies were provided with Protinex (Pfizer India) and 20% sugar solution as food and with pumpkin (*Cucurbitae moschata* Dusch.) as natural food for oviposition. The stock cultures of flies were maintained in the insect culture room/B.O.D. (biological oxygen demand) incubators with regulated temperature (25±2°C), relative humidity (70–80%) and photophase (L:D, 10:14).

The freshly emerged adult flies, procured from the stock culture, were sexed, immobilized and treated topically by applying 1 µl solution of PGR on their dorsum with a micro-applicator. In the control experiment, the flies were treated topically with the respective solvent used for preparing the stock solutions of PGRs. The treated flies were released in wire gauge cages (15×15×16 cm), and provided with Protinex, sugar solution and fresh pumpkin pieces. The pumpkin pieces on which eggs had been oviposited were changed daily, cut open, and the number of eggs in them was counted. The observations were continued until the flies died. There were six replications, each of which consisted of three pairs of adults, for all the concentrations and PGRs. A control was also run simultaneously with all the treatments of each PGR except for one or two experiments, in which tests for all concentrations could not be started at the same time; in these cases, control experiments were run with each set of treatments and the mean values of control were used for analyses. The data were tabulated for fecundity, percent fertility of eggs and total longevity. The corrected percent sterility was also calculated by applying the following formula:

$$\text{Corrected percent sterility} = \frac{\% \text{ Fertility in control} - \% \text{ Fertility in treatment}}{\% \text{ Fertility in control}} \times 100$$

The data were then subjected to statistical analysis by ANOVA, least significant difference (LSD) and linear correlation coefficient (r) in order to assess the relativity and significance of effect of PGRs on the reproductive potential and longevity of the melon fruit fly.

RESULTS

Both the fecundity and fertility of melon fruit flies were significantly reduced when the 0–1-day-old adults were treated with the four PGRs (Tables 1–4). However, the severity

of their effects was not of the same intensity. The maximum reduction in total number of eggs, percent fecundity, percent fertility and an increase in corrected percent sterility were observed in the kinetin treatment, where fecundity was reduced to $23.38 \pm 8.56\%$ with $12.72 \pm 2.81\%$ fertility even in the treatment with the lowest concentration tested, viz., 25 ppm, compared with the control; corrected percent sterility reached 85.55 ± 9.13 (Table 1). This influence of kinetin on the reproductive potential increased as its concentration was raised. The calculated r-value was -0.8794 for fecundity and -0.4045 for fertility and these values were the highest among the four PGRs. In the severity of effect on reproductive potential, kinetin was followed by coumarin and GA_3 , with $LSD_{0.01}$ values of 78.52, 135.37 and 137.36, respectively (Tables 1–3). The r-values for fecundity and fertility for coumarin treatment were -0.5595 and -0.3350 , respectively (Table 2), and those for the GA_3 treatment were -0.5611 and -0.2570 , respectively (Table 3). However, in the IAA treatment, the ANOVA test was found to be non-significant and r-values were also low, although there was a reduction of approximately 50% in the number of eggs laid (Table 4).

TABLE 1. Reproductive potential of adult female *Bactrocera cucurbitae* when treated with kinetin (values are means \pm SE)

Kinetin concentration (ppm)	Total no. of eggs/ female	% Fecundity	% Fertility	Corrected % sterility
Control	412.50 \pm 6.14	100	88.05 \pm 2.20	–
25	99.00 \pm 36.72	23.38 \pm 8.56	12.72 \pm 2.81	85.55 \pm 9.13
125	69.33 \pm 2.96	16.80 \pm 0.75	14.93 \pm 3.11	83.04 \pm 10.52
625	47.00 \pm 12.45	11.66 \pm 3.20	17.75 \pm 1.59	79.84 \pm 6.65
3125	22.00 \pm 8.96	5.51 \pm 2.26	9.45 \pm 3.45	89.26 \pm 9.76
F	67.59**	–	19.08**	–
LSD (P=0.01)	78.52	–	10.41	–
r	-0.8794	–	-0.4045	–

** , significant at 1% level.

TABLE 2. Reproductive potential of adult female *Bactrocera cucurbitae* when treated with coumarin (values are means \pm SE)

Coumarin concentration (ppm)	Total no. of eggs/female	% Fecundity	% Fertility	Corrected % sterility
Control	412.50 \pm 06.14	100	88.05 \pm 2.20	–
25	142.50 \pm 21.74	35.02 \pm 5.78	23.87 \pm 8.72	72.89 \pm 14.70
625	92.33 \pm 29.03	22.88 \pm 7.35	23.07 \pm 2.35	39.86 \pm 3.61
3125	127.83 \pm 56.89	30.04 \pm 13.29	09.34 \pm 3.41	76.38 \pm 8.52
F	19.53**	–	5.09**	–
LSD (P=0.01)	135.37	–	18.32	–
r	-0.5595	–	-0.3350	–

** , significant at 1% level.

Analysis of the data for longevity of both males and females revealed that treatment with any of the four PGRs led to reduced longevity, with coumarin and kinetin being the most potential chemicals followed by GA_3 and IAA (Tables 5 and 6). With coumarin, the longevity of both males and females was reduced to \sim one-third compared with the control, even in the 25 ppm concentration treatment, whereas in the kinetin treatment the longevity

of males only was reduced to \sim one-third and that of females was reduced to \sim half the value in the control. Longevity was further reduced when the concentration was increased in the coumarin and kinetin treatments, but correlation was not very strong: r -values for coumarin were -0.4676 (males) and -0.5274 (females), and those for kinetin were -0.4222 (males) and -0.4040 (females). These two chemicals were followed by GA_3 and then IAA in their influence.

TABLE 3. Reproductive potential of adult female *Bactrocera cucurbitae* when treated with GA_3 (values are means \pm SE)

GA_3 concentration (ppm)	Total no. of eggs/female	% Fecundity	% Fertility	Corrected % sterility
Control	412.50 \pm 6.14	100	88.05 \pm 2.20	–
25	279.50 \pm 39.14	68.53 \pm 10.49	33.05 \pm 1.15	62.46 \pm 5.32
125	170.00 \pm 57.27	40.24 \pm 13.29	30.99 \pm 2.90	64.80 \pm 4.88
625	60.17 \pm 5.99	14.48 \pm 1.23	17.11 \pm 1.46	80.56 \pm 4.93
3125	79.17 \pm 4.56	19.13 \pm 0.81	17.56 \pm 3.87	80.05 \pm 8.16
F	18.56**	–	14.24**	–
LSD ($P=0.01$)	137.36	–	10.13	–
r	-0.5611	–	-0.2570	–

** , significant at 1% level.

TABLE 4. Reproductive potential of adult female *Bactrocera cucurbitae* when treated with IAA (values are means \pm SE)

IAA concentration (ppm)	Total no. of eggs/female	% Fecundity	% Fertility	Corrected % sterility
Control	412.50 \pm 6.14	100	88.05 \pm 2.20	–
25	237.50 \pm 70.45	59.05 \pm 18.01	23.82 \pm 0.75	72.94 \pm 4.94
125	199.00 \pm 59.94	47.19 \pm 13.82	20.58 \pm 8.07	76.62 \pm 18.06
625	180.50 \pm 48.53	44.72 \pm 12.42	21.14 \pm 7.72	75.99 \pm 15.81
3125	220.50 \pm 74.91	54.84 \pm 19.00	15.97 \pm 5.83	81.86 \pm 15.43
F	2.62 (n.s.)	–	2.19 (n.s.)	–
r	-0.2469	–	-0.2659	–

TABLE 5. Longevity of adult male *Bactrocera cucurbitae* when treated with four plant growth regulators (values are means \pm SE)

Concentrations tested (ppm)	Longevity of males (days)			
	GA_3	IAA	Kinetin	Coumarin
Control	91.50 \pm 2.95	91.50 \pm 2.95	91.50 \pm 2.95	91.50 \pm 2.36
25	57.17 \pm 16.56	69.17 \pm 26.34	28.50 \pm 6.49	29.17 \pm 7.80
125	46.33 \pm 19.64	56.50 \pm 17.16	39.00 \pm 9.99	–
625	21.33 \pm 8.46	34.67 \pm 15.52	46.50 \pm 11.67	22.50 \pm 6.30
3125	30.50 \pm 10.68	21.83 \pm 9.25	15.67 \pm 4.49	33.17 \pm 7.36
F	8.68**	4.47**	19.88**	37.16**
LSD ($P=0.01$)	37.33	52.52	26.03	19.77
r	-0.4177	-0.3963	-0.4222	-0.4676

** , significant at 1% level.

TABLE 6. Longevity of adult female *Bactrocera cucurbitae* when treated with four plant growth regulators (values are means±SE)

Concentrations tested (ppm)	Longevity of females (days)			
	GA ₃	IAA	Kinetin	Coumarin
Control	87.67±2.36	87.67±2.36	87.67±2.36	87.67±2.36
25	72.67±12.50	67.50±27.03	44.00±10.71	29.50±8.52
125	60.33±19.51	51.17±24.18	33.67±11.21	–
625	33.00±10.69	54.00±17.80	31.00±10.34	24.67±6.57
3125	26.67±13.31	56.67±13.92	27.83±13.29	24.00±7.54
<i>F</i>	7.01**	0.72 (n.s.)	10.63**	41.69**
LSD (<i>P</i> =0.01)	39.40	–	30.47	18.29
<i>r</i>	-0.3549	-0.1619	-0.4040	-0.5274

** , significant at 1% level.

DISCUSSION

The critical analysis of the results revealed that all four chemicals investigated significantly reduced both fecundity and fertility, thereby inducing partial sterility in females. In addition, the treatments also shortened the longevity of melon fruit flies drastically and thus reduced the oviposition period. All the factors collectively and significantly affected the reproductive potential of this fruit fly. In the severity of effect on the reproductive potential, the influence was found to be in the following sequence: kinetin ≥ coumarin > GA₃ > IAA.

Similarly, a reduction in the reproductive potential following the application of kinetin (a kinin) has been reported in *Aphis fabae* (Scopoli), *Aulocara elliotti* (Thomas) and *Zaprionus paravittiger* (Godbole & Vaidya) (20,21,23,27). The prolongation in the developmental period and reduction in the reproductive potential with a low concentration (25 ppm) of kinetin in *Z. paravittiger* has been correlated to the anti-ageing activity of kinetin (23). The researchers hypothesized that this could be due to the fact that energy required for the production of eggs was channeled into the prolongation of younger stages, *i.e.*, developmental period.

Studies with *Drosophila melanogaster* (Meigen) (11), *Sitotroga cerealella* (Oliv.) (12), *Aphis craccivora* Koch. (9) and *Lipaphis erysimi* (Kaltenbach) (17) also demonstrated a significant reduction in the reproductive capacity following coumarin (an abscissin) treatment. In addition to these, adverse effects on the reproductive potential with another abscissin, *viz.*, ABA, have been reported in *A. elliotti* and *D. melanogaster* (26,28,29). This has been attributed to the chemical configuration of ABA, which is a sesquiterpenoid compound, derived similarly from farnesol as a juvenile hormone (JH) (26). The researchers presumed that ABA might have interfered in the metabolic pathways regulated by endogenous JH and affected the vitellogenesis. A similar effect of ABA on vitellogenesis has been reported in *Sarcophaga bullata* (2). Kinetin and coumarin – although having different chemical configurations and demonstrating a different influence in plants and perhaps a different mode of action – induce similar end results on the reproductive potential of *B. cucurbitae*.

The influence of GA₃ treatment on reproductive potential, including shortening of longevity, is also highly significant in the present study. Corroboratory influence of GA₃ on reproductive potential and longevity has been observed in *Spodoptera littoralis* (Boisd.) (19), *A. elliotti* (26) and *Z. paravittiger* (16). The chemical configuration of GA₃, a

terpenoid compound, is also similar to a JH and it was hypothesized that GA₃ interfered in the endocrinal metabolic processes involved in reproduction (1,26). However, contrary to the present findings, an improvement in the reproductive potential was reported when the nymphs of *Schistocerca gregaria* (Forsk.) and larvae of *Bombyx mori* L. were fed on medium into which low concentrations of GA₃ had been incorporated (3, 22).

In the present study IAA had a relatively low level of adverse activity on the longevity and reproductive potential of *B. cucurbitae*. Adverse activity of IAA on the reproductive potential has also been observed in *L. erysimi*, *Z. paravittiger* and *D. dorsalis* (15,18,24). This influence of IAA has been correlated to its interference with the neurosecretory system, which might affect the reproductive potential (24).

It may be concluded that different PGRs influence the well-being of various insects differently, depending on a number of factors. In the present case, of four PGRs investigated, both kinetin and coumarin – which had severe adverse effects on reproductive potential of *B. cucurbitae* at very low concentrations – should be explored further for their overall influence on the population build-up and pest status in the field. They could prove to be environmentally safe compounds for use in the management of this fly.

REFERENCES

1. Barbouche, N. and Ben Hamouda, M.H. (1987) [Effect of gibberellins, growth phyto regulators, on reproduction physiology of *Ceratitidis capitata* (Diptera:Trypetidae). *Proc. II Int. Symp. Fruit Flies* (Colymbari, Crete), pp. 103-113.
2. De Man, W., De Loof, A., Briers, T. and Huybrechts, R. (1981) Effect of abscisic acid on vitellogenesis in *Sarcophaga bullata*. *Entomol. Exp. Appl.* 29:259-267.
3. Ellis, P.E., Carlisle, D.B. and Osborne, D.J. (1965) Desert locusts: Sexual maturation delayed by feeding on senescent vegetation. *Science* 149:546-547.
4. Greany, P.D. (1989) Host plant resistance to tephritids: An under-exploited control strategy. *in: Robinson, A.S. and Hooper, G. [Eds.] World Crop Pests. Vol. 3A*, pp. 353-362. *Fruit Flies - Biology, Natural Enemies and Control*. Elsevier Science Publ., Amsterdam, the Netherlands.
5. Greany, P.D., McDonald, R.E., Shaw, P.E., Schroeder, W.J., Howard, D.F., Hatton, T.T. *et al.* (1987) Use of gibberellic acid to reduce grapefruit susceptibility to attack by the Caribbean fruit fly *Anastrepha suspensa* (Diptera: Tephritidae). *Trop. Sci.* 27:261-270.
6. Gupta, J.N., Verma, A.N. and Kashyap, R.K. (1978) An improved method for mass rearing of melon fruit fly *Dacus cucurbitae* Coquillett. *Indian J. Entomol.* 40:470-471.
7. Kaur, R. and Rup, P.J. (1999) Evaluation of gibberellic acid (PGR) against immature stages of *Bactrocera cucurbitae* (Coquillett), the melon fruitfly. *J. Insect Sci.* 12(1):9-14.
8. Kaur, R. and Rup, P.J. (2000) The bioregulatory influence of indole-3-acetic acid (PGR) on the melon fruitfly, *Bactrocera cucurbitae* (Coquillett) (Diptera:Tephritidae). *Int. J. Trends Life Sci.* 15(2):103-108.
9. Mansour, M.H., Dimetry, N.Z. and Rofald, T.S. (1982) The role of coumarin as a secondary plant substance in the food specificity of the cowpea aphid *Aphis craccivora* Koch. *Z. Angew. Entomol.* 92:151-157.
10. McDonald, R.E., Greany, P.D., Shaw, P.E., Schroeder, W.J., Hatton, T.T. and Wilson, C.W. (1988) Use of gibberellic acid for Caribbean fruit fly (*Anastrepha suspensa*) control in grapefruit. *Proc. 6th Int. Citrus Congr.* (Tel Aviv, Israel), pp. 1147-1152.
11. Nakajima, S. and Kawazu, K. (1980) Coumarin and eupoinin, two inhibitors for insect development from leaves of *Eupatorium japonicum*. *Agric. Biol. Chem.* 44:2893-2899.
12. Pandey, G.P. and Teotia, T.P.S. (1980) Laboratory studies on chemosterilization of Angoumois grain moth, *Sitotroga cerealella* (Oliv.): Screening of 33 compounds by pupal treatments. *Indian J. Entomol.* 42:1-15.
13. Remadevi, O.K., Magadam, S.B., Nair, K.S. and Datta, R.K. (1998) Plant growth hormones and silk. *Indian Text. J.* 108:78-80.
14. Rup, P.J., Arora, K. and Kaur, R. (2000) Exploration of kinetin for its effects on the development on melon fruit fly, *Bactrocera cucurbitae* (Coquillett). *in: Thukral, A.K. and Virk, G.S. [Eds.] Environmental Protection*. Scientific Publ., Jodhpur, India. pp. 304-310.
15. Rup, P.J. and Dhillon, M.K. (1999) Morphogenetic and biochemical responses of the mustard aphid, *Lipaphis erysimi* (Kalt.) (Homoptera: Aphididae), to indole-3-acetic acid (IAA). *J. Aphidol.* 13:31-38.

16. Rup, P.J. and Kalia, S. (1993) Effect of gibberellic acid on the development of banana fruit fly, *Zaprionus parvittiger* (Godbole and Vaidya) (Drosophilidae:Diptera). *Pest Manage. Econ. Zool.* 1:27-31.
17. Rup, P.J., Kaur, P. and Sohal, S.K. (1998) Influence of coumarin (a secondary plant compound) on the morphology and biochemistry of the mustard aphid, *Lipaphis erysimi* (Kalt.). *J. Environ. Biol.* 19:251-257.
18. Rup, P.J., Kumari, S. and Sohal, S.K. (1997) Morphogenetic responses of *Zaprionus parvittiger* to indole acetic acid. *in: Grover, I.S. and Thukral, A.S. [Eds.] Environment and Development.* Scientific Publ., Jodhpur, India. pp. 289-293.
19. Salama, H.S. and El-Sharaby, A.M. (1972) Gibberellic acid and β -sitosterol as sterilants of the cotton leafworm, *Spodoptera littoralis* Boisduval (Lepidoptera:Noctuidae). *Experientia* 28:413-414.
20. Scheurer, S. (1976) The influence of phytohormones and growth regulating substances on insect development processes. *Symp. Biol. Hung.* 16:255-259.
21. Scheurer, S. and Aschermann, S. (1974) [The influence of natural and some synthetic plant regulators on the reproductive activity of *Aphis fabae* sucking plant parts.] *Folia Entomol. Hung.* 27:221-226 (in German).
22. Shantakumari, M., Sreedhara, V.M. and Fletcher, R.A. (1989) Gibberellic acid improves the commercial characteristics of silkworms. *Sericologia* 29:347-351.
23. Sharma, S.P., Kaur, J. and Rattan, S.I.S. (1997) Increased longevity of kinetin-fed *Zaprionus* fruit flies is accompanied by their reduced fecundity and enhanced catalase activity. *Biochem. Mol. Biol. Int.* 41:869-875.
24. Thakur, J.N. and Kumar, A. (1984) Effects of 3-indole acetic acid on the fertility of fruit fly, *Dacus dorsalis* Hendel (Diptera: Tephritidae). *Natl. Acad. Sci. Lett. (India)* 7:197-199.
25. Thakur, J.N. and Mann, S.K. (1982) Effects of indole-3-acetic acid on the histology of gonads and their development in *Dacus dorsalis* Hendel. *Experientia* 38:490-492.
26. Visscher, S.N. (1980) Regulation of grasshopper fecundity, longevity and egg-viability by plant growth hormones. *Experientia* 36:130-131.
27. Visscher, S.N. (1982) Plant growth hormones affect grasshopper growth and reproduction. *in: Visser, J.H. and Minks, A.K. [Eds.] Proc. 5th Int. Symp. Insect-Plant Relationships* (Wageningen, Netherlands), pp. 57-62.
28. Yesilada, E. and Bozcuk, A.N. (1995) [The effects of ABA and kinetin on the fecundity of *Drosophila melanogaster*.] *Turk. J. Biol.* 19:37-44 [in Turkish].
29. Yesilada, E. and Bozcuk, A.N. (1996) [The effects of ABA and Kinetin on the developmental period of *Drosophila melanogaster*.] *Turk. J. Biol.* 20:29-35 [in Turkish].