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RESEARCH ARTICLE

EFFICACY OF JHA, FENOXYCARB ON EGG HATCHABILITY AND POST EMBRYONIC DEVELOPMENT OF SPODOPTERA MAURITIA BOISD.(LEPIDOPTERA: NOCTUIDAE)

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Abstract

The egg hatchability of *Spodoptera mauritia* was studied by exposing freshly laid (0–24 h), following with (24-48h) and to (48–72 h) eggs to different concentrations (5×10^{-5} – 1.0 µM/µl) of juvenile hormone analogue (JHA), fenoxycarb. Dosage dependent response was observed after the treatment. The response was dependent on the age of eggs and also found higher doses resulted in maximum reduction in hatching. Treatments of eggs caused significant differences in egg mortality between control and treatments, with LD_{50}= 0.002 µM, LD_{90}= 0.048 µM and LD_{50}= 0.002 µM, LD_{90}= 0.071 µM for (0-24 h) and (24-48h) respectively. In the case of delayed treatment (48h-72h) eggs hatched as that of control. The result found that freshly laid eggs were more sensitive than those treated 2 or 3 days old eggs. Fenoxycarb applied eggs has delayed effects on postembryonic development. Here, we observed that the resulting larvae in earlier stages showed some effects such as increased larval duration, formation of intermediates and subsequent death. Therefore, JHA Fenoxycarb has great potency in suppressing the population by affecting hatchability of eggs of *S mauritia*, and thus provides a useful tool for pest management.

Introduction:-

In insects, the process of growth and development is directly or indirectly regulated by the three, major circulating hormones Prothoracicotropic hormone (PTTH), Ecdysone and Juvenile hormone (JH). Hormone analogues and mimics can artificially regulate insect development, and the discovery of these mimics have immensely helped in pest management by protecting plants from insect pests, limiting resistance to pest control agents and protecting beneficial organisms. What makes IGRs different from the common insecticides is the fact that they disrupt the normal activity of endocrine system thereby influencing the development, metamorphosis and reproduction of target insects. IGRs have their unique mode of action, as a result they have also been considered less harmful to beneficial insects as when compared with conventional neurotoxicants [1,2]

Since early 1970s, many JH analogues (juvenoids) have been tested for insecticidal activity [3] and most of these early analogues had a basic terpenoid structure that resembled JHs. Recently, highly active compounds like Fenoxycarb, Pyriproxyfen and Diofenolan have less similarity to JH (aromatic non- terpenoidal JH analogs) have been synthesized [4,5]. Other than morphogenetic activities, Juvenile hormone and analogues were also found to

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render the treated insects sterile or less fecund. Treated eggs failed to hatch directly [6,7] or indirectly [8], due to the inhibition of embryonic development, or when hatched, the progeny suffered latent effects.

The insect growth regulator, fenoxycarb (ethyl [2- (4-phenox)'phenoxy) ethyl] carbamate) – is a broad spectrum juvenile hormone analogue (JHA) which is effective against many insect pests like scales, fleas, mosquitoes, houseflies, stored product insects, psyllids and many Lepidopteran species [9,10,11,12]. Further, it is noted that in these insect species the fenoxycarb treatments were found to disrupt embryonic development and reduced egg hatchability. Recently, a study reporting the sublethal effects of fenoxycarb on *Plutella xylostella*, found that there is a significant prolongation on the developmental time of eggs, larvae, and pupae in the offspring generation [13]. Eventually, this might lead to increase exposure risk to natural enemies [14]. The studies on the exact mode of action of fenoxycarb on *S. mauritia* eggs are still unknown. So, the present study was undertaken for the better understanding of the mode of action of fenoxycarb on the embryogenesis of *S. mauritia* eggs.

Materials and Methods:-

Insect Culture: - Insects needed for the study were obtained from a laboratory culture of *Spodoptera mauritia* maintained at room temperature RH 90±3% and 10:14 light: dark photo period. The culture was initiated by collecting 5th and 6th - instar larvae and pupae from paddy fields at the time of outbreak. Upon emergence, healthy adult males and females were collected for the study; mating pairs were kept in separate glass beakers with a cloth top and fed with diluted honey. Egg batches were collected every 24hrs.

Egg Toxicity Bioassay:-

In the case of egg toxicity bioassay, eggs of known ages were treated with different concentrations of fenoxycarb. The selected egg batches were dipped in a series of varying concentrations of Fenoxycarb solutions dissolved in acetone, for 5 sec at different intervals after oviposition, i.e. 1, 2 and 3 days. Acetone treated set were taken as vehicle control and untreated set were taken as control. The treated eggs were air dried in Petri dishes for 5 mts. Then, the eggs were transferred to rearing beakers and their hatching rate was recorded daily. The eggs normally hatched in about 3-4 days after oviposition and unhatched ones were counted as dead. Hatched larvae from each treatment were reared in glass beakers and fed with tender leaves of *Ischaemum aristatum*. Hatchability rate, survival rate, mortality during larval stage, percentage of pupation and adult emergence were recorded.

Statistical analysis:-

Statistical analysis was performed with the help of MS-excel and IBM SPSS (20.0 versions). The data were expressed as Mean ± SE and the statistical significance of differences between individuals was determined using one way ANOVA test. The level of significance of each experiment was stated to be significant at ($P< 0.05$). The significance of difference in different doses was tested using Duncan's Multiple Range Test (DMRT) using Post Hoc Multiple Comparison Test. Differences were indicated by using the alphabets a, b, c, d, etc.

The Mortality data was corrected by using Schneider-Orelli's Formula and further to obtain the lethal dose (LC_{50} & LC_{90}), the corrected data were analysed by probit analysis [15]

Schneider-Orelli's Formula:-

$$\text{Corrected \%} = \left[\frac{\text{Mortality \% in treated plot - Mortality \% in control plot}}{\text{100- Mortality \% in control plot}}\right] \text{x } 100$$

Results:-

The effect of topical applications of the JH analogue, Fenoxycarb on the eggs of *S. mauritia* were determined. It is clear from the results summarized in Table 1, that the eggs at an early stage of embryogenesis (0–24 h old) were more sensitive to the compound than older eggs (48–72 h old) that determined the egg hatchability rate (Table 1). None of the eggs treated with 1µM and 0.5 µM hatched in 24hr aged eggs; effects were noticeable even at 0.1 µM which caused about 92 % reduction in egg hatchability. When treatment was postponed to the second day, sensitivity was decreased and on the third day, eggs hatched similar to that of control groups. The observed data showed an ovicidal activity of the compound, and demonstrated a progressive decline in egg hatchability on

application with increasing rates of fenoxycarb. The calculated LC_{50} and LC_{90} values are 0.002 µM and 0.072 µM, respectively (Table 2).

Table 1:-Eggs hatchability of *S. mauritia* after treatment with Fenoxycarb at different ages

Concentrations (µM)	Egg hatchability (%) ($\bar{x}$±SE)	
	0 - 24 hr	**24 - 48 hr**
Control	99 ± 0.41^{j}	97 ± 0.25^{k}
Vehicle Control	97 ± 0.41^{j}	97.75 ± 0.47^{jk}
0.00005	93.75 ± 1.31^{i}	96.75 ± 0.49^{j}
0.0001	79.75 ± 0.85^{h}	86.25 ± 0.95^{i}
0.0005	66.5 ± 0.64^{g}	74.25 ± 0.35^{h}
0.001	58.25 ± 0.85^{f}	60.5 ± 0.64^{g}
0.005	30.25 ± 0.62^{e}	31.75 ± 0.62^{f}
0.01	23.5 ± 0.64^{d}	25.5 ± 0.65^{e}
0.05	13.75 ± 0.85^{c}	15.25 ± 0.85^{d}
0.1	7.75 ± 0.62^{b}	10.25 ± 0.85^{c}
0.5	0^{a}	4.5 ± 0.65^{b}
1.0	0^{a}	0^{a}

Means in the same column followed by the same letters do not differ significantly at P <0.05 (Duncan test). 4 replicates were treated at each concentration.

Table 2:-Lethal Concentrations against *S. mauritia* eggs after topical treatment with Fenoxycarb at different periods of oviposition

		Egg Mortality	
		0 - 24 hr	24 - 48 hr
LD_{50} (µM)		0.002	0.002
95% confidence limit	LFL	0.001	0.002
	UFL	0.002	0.004
LD_{90} (µM)		0.48	0.071
95% confidence limit	LFL	0.033	0.041
	UFL	0.075	0.139
χ^2 (df=8)		9.371	12.108
Variance (F)		5.203	5.401
Regression Equation		y = 75.57 (x) + 41.566	y = 77.048 (x) + 38.892
R^2		0.342	0.351
P value		0.046	0.042

LFL = Lower Fiducidal Limit, UFL = Upper Fiducidal Limit, χ2–Chi-square value, df - degrees of freedom

The purpose of this study has been to describe the progressive morphological effects of JHA on the embryos of *S.mauritia*. Hatched eggs from the treated group larvae grew normally, but later their metamorphosis was blocked and they developed into intermediates or extra larval instars. Treatment during the early hours (0-24 hrs) was associated with higher incidence of developmental anomalies in larval life (Figs. 1&2), with the changes such as the formation of non-viable first instar larvae which failed to feed and normal larvae died at one of the succeeding moults. All larval intermediates were dead prior to metamorphosis and those which showed normal larval development completed the metamorphosis. The data presented in (Table 3) indicate that about 58.75% adults emerged from egg masses treated with 0.01 µM concentration of fenoxycarb, and among them 8.75 % were abnormal.

Fig 1:-

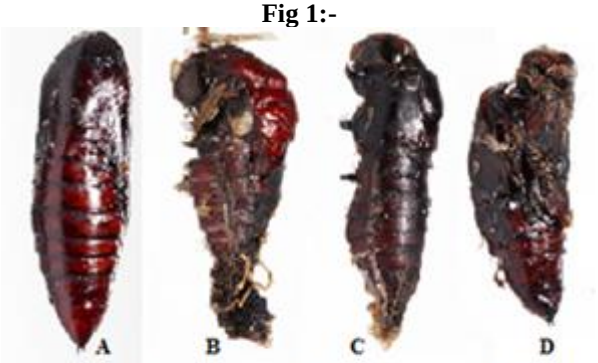

Fig. 1. A - Normal pupa, B - Abnormal pupa unable to molt, C - larval pupal intermediate, D - Abnormal pupa.

Fig 2:-

Fig. 2. A - Normal adult, B, C, D - Abnormal adults

Table 3:-The latent effects of post treatment with Fenoxycarb on the eggs of *S. mauritia* at 0-24hr after egg laying

Life stages		Concentrations (µM)						
		Control	Vehicle Control	0.0001	0.001	0.01	0.1	1.0
Larval Mortality (%) ($\bar{x}$±SE)	1st Instar	11.75 ± 1.4^{b}	14.25 ± 2.1^{b}	23.5 ± 2.53^{c}	28 ± 2.74^{c}	35 ± 1.47^{d}	54.25 ±3.57^{e}	-
	2nd Instar	8.25 ± 1.49^{b}	9.25 ± 1.38^{b}	14.25 ± 1.49^{c}	15.75 ± 1.3^{cd}	18.5 ± 1.3^{de}	21.25 ± 1.7^{e}	-
	3rd Instar	7.25 ± 0.85^{b}	7.75 ± 1.49^{b}	10.25 ± 1.11^{b}	10 ± 1.08^{b}	15 ± 1.08^{c}	17.75 ±1.31^{c}	-
	4th Instar	5.5 ± 0.64^{b}	5.25 ± 0.85^{b}	8 ± 0.41^{bc}	10.75 ± 1.4^{cd}	12 ± 1.47^{d}	12.75 ±1.11^{d}	-
	5th Instar	4.25 ± 0.47^{b}	4.5 ± 0.64^{b}	6.5 ± 0.64^{bc}	8.75 ± 0.85^{cd}	10.3 ± 1.5^{d}	13 ± 1.08^{e}	-
	6th Instar	4.5 ± 0.64^{b}	5.5 ± 0.64^{b}	6.25 ± 0.64^{b}	6.75 ± 0.85^{b}	10 ± 1.49^{c}	10.5 ± 1.08^{c}	-
Pupation (%) $\bar{x}$		97	96	93.5	87.5	87	71.75	-
Abnormal Pupae (%) $\bar{x}$		2.25	2.25	8.75	14.75	31	76	-
Adult Emergence(%) $\bar{x}$		98	96.75	92.75	84.75	58.75	-	-
Abnormal Adult (%) $\bar{x}$		0	0.25	0.5	1.5	8.75	-	-

$\bar{x}$±SE = Mean ± Standard Error, Means in the same column followed by the same letters do not differ significantly at P <0.05 (Duncan test). 4 replicates of were treated at each concentration.

Discussion:-

Earlier studies revealed that exogenous topical applications of JH and JHAs block embryonic development in *Pyrrhocoris apterus* [16], *Hylaophora cecropia* [7], *Schistocerca gregaria* [17], *Choristoneura fumiferana* [18],

Thermobia domestica [19], *Cydia pomonella* [20], and *Drosophila melanogaster* [21]. In all these cases, the treated insect embryos continued development to a certain stage and lead to stoppage of the individuals followed by death. These results agree fully with the current study, that exogenous topical applications of JHA, fenoxycarb was more effective in suppressing hatching of eggs in *S. mauritia* and moreover, increasing rates of fenoxycarb concentrations resulted in progressive decrease of egg hatchability. The ability of juvenile hormone to block the embryonic development of insects was discovered by Slama and Williams [16] in their study on sensitivity of *Pyrrhocoris apterus* eggs to the juvenile hormone analogue 'Juvabione." They found that adult females exposed to a crude preparation of juvabione, oviposited eggs failed to hatch. The same result was obtained when the hormonal analogue was topically applied to freshly laid eggs of *S.mauritia*. In both cases, embryonic development proceeded to a certain stage and finally culminated to death. Our results agree with the studies on the application of pyriproxyfen resulted in greater suppression of egg hatching and inhibition of adult emergence and further pyriproxyfen also reduced female fecundity [22].

From the study, we observed that the eggs of *S. mauritia* at an early stage of embryogenesis (0–24 h old) were more sensitive to the compound than older eggs (48–72 h) and proved that the age of egg was determined with the egg hatchability rate. These findings were confirmed in another detailed study of two species of silkworms [6, 23], where the eggs were found to be maximally sensitive just before or immediately after oviposition. Majority of eggs hatched as normal larvae, when the hormonal treatment was postponed until the 48th hour of incubation. These findings are in congruent with those obtained with glassy-winged sharpshooter, *Homalodisca vitripennis* by [24].

It has been reported that JHAs were more effective at the starting stage of metamorphosis and embryogenesis, such as freshly ecdysed last larval and pupal instars and freshly deposited eggs [4, 25]. Thus, the present study observed a dose dependent reduction in the hatchability of eggs, and it may be due to disrupted embryogenesis when young eggs are treated with fenoxycarb. The observations of the current study are coinciding with the observation of fenoxycarb application to eggs, which proved significant ovicidal effect on *C. rufilabris* eggs [26]. Similarly, studies on the treated insect eggs showed disruption of the blastoderm with associated cellular and organelle disruption [4].

Several progressive morphological defects were observed following the treatments of JHA on embryos of *S.mauritia*. Such as, the larvae grew normally, but later their metamorphosis was blocked and they developed into intermediates and extra larval instars. Similar observations were recorded by few authors [6, 27, 7, 28]. These results are clear evidences for the negative influence of JHA, on egg hatchability and postembryonic development of insects.

Conclusion:-

The above finding concludes that the early-developing egg stages of *S.mauritia* are highly susceptible to Fenoxycarb, also it should be mentioned that, this JHA could cause direct lethal effects such as mortality on eggs, production of larval pupal intermediates and abnormal adults. These effects were observed in laboratory conditions. Further, it is understood that the combination of lethal and sublethal effects of Fenoxycarb could produce significant negative effects on the population dynamics of *S.mauritia*. On the basis of our findings we conclude that Fenoxycarb has higher potential for controlling *S.mauritia*.

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References:-

1. Ellsworth, P and Martinez-Carillo J, IPM for *Bemisia tabaci*: a case study from North America. Crop Prot . 2001, 20,853–869. https://www.sciencedirect.com/science/article/pii/S0261219401001168
2. Naranjo, S.; Ellsworth, P.C. and Hagler, J.R. Conservation of natural enemies in cotton: role of insect growth regulators in management of *Bemisia tabaci*. Biol Cont. 2004, 30, 52–72. https://www.sciencedirect.com/science/article/pii/S1049964403002081?via%3Dihub
3. Sehnal, F. Action of juvenoids on different groups of insects. In: Gilbert LI [ed.] The Juvenile Hormones. Plenum Press, New York. 1976, NY. pp. 301-321. https://link.springer.com/chapter/10.1007/978-1-4684-7947-8_21
4. Dhadialla, T. S.; Carlson, G. R. and Le, D. P. New insecticides with ecdysteroidal and juvenile hormone activity. Annual Review of Entomology. 1998, 43: 545 -69. [PubMed] [Google Scholar]
5. Dhadialla, T.S.; Retnakaran, A. and Smagghe, G. Insect growth- and developmental-disturbing insecticides. In: Gilbert LI, Iatrou K & Gill SK (eds), Comprehensive Molecular Insect Science, vol. 6. Elsevier, Oxford. 2005, pp. 55-116. https://www.researchgate.net/publication/313044176_Insect_growth_regulators
6. Riddiford, L. M.; and Williams, C. M. The effects of juvenile hormone analogues on the embryonic development of silkworms. Proc. N&l. Acad. Sci. U.S. 1967, 57, 595-601. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC335550/
7. Riddiford, L. Effects of juvenile hormone on the programming of post-embryonic development in the eggs of the silkworm, *Hyalophora cecropia*. Dev. Bioi.. 1970, 22, 249-263. https://www.sciencedirect.com/science/article/pii/0012160670901533
8. Masner, P.; Slama, K.; Zdarek, K.; Landa, V. Natural and synthetic materials with insect hormone activity X. A method of sexually spread insect sterility. J. Econ. Entomol. 1970, 63, 706-710. https://academic.oup.com/jee/article-abstract/63/3/706/898235?redirectedFrom=PDF
9. Dorn, S.;. Frischknecht, M. L.; Martinez, V.; Zurfluh, R. and Fischer, U. A novel nonneurotoxic in secticide with a broad activity spectrum. Zcitschrift fur Pflanzenkrankheitcn und Pflanzen schutz. 1981,88, 269-275 . https://www.jstor.org/stable/43214853
10. Masner, P.; Dorn, S.; Vogel, W. ; Kalin, M.; Graf, O. and Gunthart, E. Types of responses of insects to a new IGR and to proven standards. Scientific Papers of the Institute of Organic and Physical Chemistry of Wroclaw Technical University. 1981, No. 22, Conference 7: 809-818. [Google Scholar]
11. Charmillot, P. 1. Les regulateurs de croissance d'insectes (RCI), mimiques de hormone juvenile, en tent que moyen de lutte morphogenetique et ovicide contre les tordeuses des vergers. Entomologia Experimentalis et Applicata. 1989, 57: 59-69. https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1570-7458.1989.tb01214.x
12. Gordon, R. Toxic effects of a commercial formulation of fenoxycarb against adult and egg stages of the eastern spruce budworm, *Chorisloneura fumiferana* (Clements) (Lepidoptera: Tortricidae). The Canadi an Entomologist. . 1995, 127: 1-5. https://booksc.xyz/book/38153210/82f330
13. Mohammad Mahmoudvand and Saeid Moharramipour. Sublethal Effects of Fenoxycarb on the *Plutella xylostella* (Lepidoptera: Plutellidae). J. Insect Sci. 2015, 15(1): 82; DOI: 10.1093/jisesa/iev064 https://www.hindawi.com/journals/psyche/2015/453701/
14. Charleston, D. S. Integrating biological control and botanical pesticides for management of *Plutella xylostella*. Wageningen University, TheNetherlands. 2004. [Google Scholar]
15. Finney, DJ. Probit analysis: a statistical treatment of the sigmoid response curves, Cambridge University Press, London, 3rd edn. 1971, 333.
16. Slama, K. and Williams, C. 'Paper factor' as an inhibitor of the embryonic development of the European bug, *Pyrrhocoris apterus*. Nature (London) 210. 1966, 329-330. https://www.nature.com/articles/210329a0
17. Novak, V. Morphogenetic analysis of the effects of juvenile hormone analogues and other morphogenetically active substances on embryos of *Schistocerca gregoria* (Forskal). J. Embryo!. Exp. Morphol., 21. 1969, 1-21.
18. Retnakaran, A. Blocking of embryonic development in the spruce budworm, *Choristoneura fumiferana* (Lepidoptera: Tortricidae), by some compounds with juvenile hormone activity. Can. Entomol. , 102. I970, I592-1596. https://doi.org/10.4039/Ent1021592-12
19. Rohdendorf, E. and Sehnal, F. Inhibition of reproduction and embryogenesis in the firebrat, *Thermobia domestica*, by juvenile hormone analogues. J. Insect Physiol, I9 .1973, 37-56. https://doi.org/10.1016/0022-1910(73)90220-5
20. Gelbic, I. and Sehnal, F. Effects of juvenile hormone mimics on the codling moth *Cydia pomonella* (L.) (Lep., Olethreutidae). Bull. Entomol. Res., 63. 1973, 7-16.https://doi.org/10.1017/S0007485300050847
21. Smith, R. and Arking, R. The effects of juvenile hormone analogues on the embryogenesis of *Drosophila melanogaster*. J. Insect Physiol, 2 I. 1975, 723-732. https://doi.org/10.1016/0022-1910(75)90004-9

22. Dhana Raj Boina; Michael, E Rogers; Nian Wang and Lukasz L Stelinski. Effect of pyriproxyfen, a juvenile hormone mimic, on egg hatch, nymph development, adult emergence and reproduction of the Asian citrus psyllid, *Diaphorina citri* Kuwayama. Pest Manag Sci. 2010, 66: 349–357. https://www.ncbi.nlm.nih.gov/pubmed/19937968
23. Benskinj and Vinson, S. B. Factors agecting juvenile hormone analogue activity in the tobacco budworm. J. Econ. Entomol. 1973, 66, 15-20. https://doi.org/10.1093/jee/66.1.15
24. Prabhaker, N. and Toscano, N.C. Toxicity of the insect growth regulators, buprofezin and pyriproxyfen, to the glassy-winged sharpshooter, *Homalodisca coagulata* Say (Homoptera: Cicadellidae). Crop Prot. 2007, 26:495–502. https://doi.org/10.1016/j.cropro.2006.04.019
25. Tunaz, H. and Uygun, N. Insect growth regulators for insect pest control. Turk J Agr Forestry. 2004, 28: 377-387.
26. Tong-xianliu and Tian-ye chen. Effects of the insect growth regulator Fenoxycarb on immature *Chrysoperla rufilabris* (Neuroptera: Chrysopidae). Florida Entomologist. 2001, 84(4), 628-633. https://pdfs.semanticscholar.org/0cba/037b53acc6260a33ecc203930f0ddf6c250a.pdf
27. Willis, J.H. The programming of differentiation and its control by juvenile 113:- rnone in saturniids. J. Ernbryol. exp. Morph. 1969, 22, 27-44. https://www.ncbi.nlm.nih.gov/pubmed/5804907
28. Riddiford, L and Trumajn,W . Delayed effects of juvenile hormone on insect metamorphosis are mediated by the corpus allatum. Nature. 1972, 237, 458. https://booksc.xyz/book/10436838/559546.