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Impact of Methyl Farnesoate in the Regulation of Molting and Reproduction in the Tropical Penaeid Prawn *Penaeus monodon*

[1]*Y. Suneetha,* [2]*P. Naga Jyothi and* [3]*M. Srinivasulu Reddy*

[1]Department of Zoology, Sri Venkateswara University, Tirupati-517 502. A.P., India
[2]Department of Fishery Science and Aquaculture,
Sri Venkateswara University, Tirupati-517 502. A.P., India
[3]Department of Zoology, SVU PG Center, Kavali, A.P., India

Abstract: The phenomenon of growth and reproduction are essentially linked to the molting cycle in crustaceans. Hence a clear knowledge about molting and reproduction are essential. The best way of demonstration for stimulation of maturation phenomenon in crustaceans is by a non-surgical procedure in either laboratory or in natural conditions. The influence of methyl farnesoate (MF) in the regulation of molt and gonad development in tropical penaeid prawn *Penaeus monodon* was investigated. Injection of methyl farnesoate into female and male prawns significantly ($P<0.001$) increased mean oocyte diameter and testicular follicle diameter as well as mean gonad indices and also accelerated the molting. These results provide strong evidence that methyl farnesoate is involved in the control of both molting and reproduction in *Penaeus monodon*.

Key words: Methyl farnesonate · Molting · Reproduction · Penaeid prawn · *Penaeus monodon*

INTRODUCTION

The phenomenon of growth and reproduction are essentially linked to the molting cycle in crustaceans. Regulation of molting and reproduction in crustaceans involves the steroidal molting hormones, the ecdysteroids [1, 2] and the sesquiterpenoid methyl farnesoate (MF) [3]. This process is under the negative control of molt inhibiting hormone (MIH) [4]. Likewise, MF is synthesized and secreted from mandibular organs (MOs) of crustaceans and is also under the negative control of mandibular organ inhibiting peptide hormone (MOIH), derived from the X-organ-sinus gland complex in the eyestalk [5, 6]. The physiological role of this compound has been subject to debate in recent years [7, 8]. Methyl farnesoate is known to be involved in the regulation of molting [9, 10], some aspects of reproduction [11-13], morphogenesis [13, 14] and general protein synthesis [15].

It is very much needs to carry out study about the molt and reproduction stages of edible crustaceans which will be useful in hatchery and farming operations. The common practice of inducing precocious reproduction in crustaceans is by eyestalk ablation [16]. This procedure though is effective in inducing reproduction in many commercially important crustacean species, but mortality of animals either at the time of surgery or after operation as well as the permanent damage are drawbacks. Moreover, in several species of crustaceans, this technique fails to maintain seed quality and larger quantity [17]. Consequently, several means and ways were propagated to stimulate gonad development and maturation in crustaceans. The best way of demonstration for stimulation of maturation phenomenon in crustaceans is by a non-surgical procedure in either laboratory or in natural conditions. An elaborate programme to induce molting and reproduction in selected edible crustaceans has been undertaken in this laboratory. The present study has been made to know the effect of injection of MF on molt and reproduction in the tropical penaeid prawn *Penaeus monodon* (Fabricius).

MATERIALS AND METHODS

The tropical penaeid prawn, *Penaeus monodon* Fabricius (1798), were collected from culture ponds in and around Kavali, (Andhra Pradesh, South India) and maintained in the Laboratory at 25±1°C in fresh water tubs. They were acclimatized to laboratory conditions (12:12 L/D), salinity of 15±1 ppt for at least 4-5 days before being used in experiments.

Trans, Trans-methyl farnesoate was dissolved in 95% ethanol, which was the solvent vehicle. The concentration

Corresponding Author: Dr. Y. Suneetha, Department of Zoology, Sri Venkateswara University, Tirupati-517 502, A.P., India

used in the experiment was equivalent to 2 ng per ml hemolymph. Sujay Kumar [18] found that the hemolymph volume (ml) of the prawn *P. monodon* is 20% of body mass. Assuming that the prawn used in these experiments had 5.5-6.0 ml of hemolymph, then the final concentration of Methyl Farnesoate used will be approximately 2 ng per ml hemolymph, which is the physiological concentration [19].

One hundred and fifty prawns *P. monodon* were divided into 6 groups of 25 animals each. Groups with odd numbers are females and even numbers are males. Group 1 and 2 served as initial controls and prawn in these groups, which received no treatment were sacrificed on the first day of experiment. Group 3 and 4 received 10 μl of Crustacean saline (containing 1μl 95% ethanol) through the arthrodial membrane of the coxa of the walking legs and served as concurrent controls. Prawns, group 5 and 6, received methyl farnesoate at a dose of 15 ng/ prawn in 10 μl volume. Injections were given on the 1st, 7th, 14th, 21st day and the prawns were sacrificed on day 22. No deaths were occurred in the control or in the experimental groups. Only intemolt prawns in the weight range of 20±2 g were used in the present study.

The prawns were immobilized by chilling on ice for 10 min. The body weights of the prawns were determined. The reproductive organs were isolated, immediately placed in ice-cold Crustacean Physiological saline [20] to scrap off adhesive tissue. The organs were removed from the saline and lightly blotted with the paper towels, wet weights were recorded. The Reproductive stages in the prawns were identified according to AIMS [21] and the molt stages were observed using setal development in the mastigobranch of third maxillipede as described by Drach and Tehenigovtzeff [22].

The gonadal indices were determined using the standard formula:

$$\text{Gonadal Index} = \frac{\text{Wet weight of the gonad (g)}}{\text{Wet weight of the prawn (g)}} \times 100$$

Reproductive organs were isolated from control prawns. They were gently rinsed with a Physiological saline solution (0.9% NaCl) to remove adhesive tissues and fixed in aqueous Bouin's fluid for 24 h. The fixative was removed by washing through running tap water overnight. After dehydrating through a graded series of alcohols, the reproductive organs were cleared in Xylene, embedded in Paraffin wax (56-58°C). Sections were cut at 7 μm thickness and stained with Hematoxylin [23] and counter stained with Eosin. The diameters of randomly chosen testicular follicles and oocytes were measured using an ocular micrometer on a compound microscope. The oocytes were observed for detection of vitellogenesis.

Results obtained were subjected to the following statistical analyses. The means of different size glands was analyzed by one-way ANOVA using Student-Newman-Keul's (S-N-K) test.

RESULTS AND DISCUSSION

All the control prawns were in the intermolt stage. The prawns groups served as concurrent control were also in the intermolt stage, after the completion of experiment. Most of the prawns, which received the MF-Injection entered into the premolt stage. Among them, three females were molted (12%), after the 4th dose, 16% of prawns entered into Do stage, 8% entered into $D_1^{\ I}$ stage, 20% enter into D_1^{II}, 8% entered into $D_1^{III}$and 12% entered into D_2 (Table 1). These data depict the effect of MF on Ovarian stage and ovarian colour in female prawn *Penaeus monodon* kept in 15 ppt media.

In the prawn, *P. monodon*, the previtellogenic ovary is translucent to opaque white. Whereas by the end of the experiment (22 days) the oocytes of the concurrent control prawns had enlarged slightly, but not significantly from control oocytes showing that only a small amount of ovarian growth had occurred during the experimental period in the prawns injected with ethanol alone. The ovaries of the concurrent control prawns are white in colour. During vitellogenesis, the colour of the ovary changes from pale yellow/ light yellow in stage-I to dark yellow in stage-II and it becomes dark yellow/ brown in stage-III and in stage-IV. The ovaries of prawns that received MF are in vitellogenic stage-I (32%), vitellogenic stage-II (16%), vitellogenic stage-III (40%) and vitellogenic stage-IV (4%) whereas two prawns were molted (8%) (Table 1). Maturation of the ovary also included an increase in size and increase in diameter of oocytes and increases in diameter due to yolk deposition. Due to MF administration, there is a significant ($P<0.001$) increase in the ovarian index in prawns. The mean oocyte diameter was significantly increased in MF injected prawns from the concurrent control as well as from the initial control prawns. The ovarian index (0.41±0.01) of vitellogenic stage-I prawns was significantly greater than

Table 1: Effect of injection of methyl farnesoate on molting and reproduction in female prawn, *Penaeus monodon*

	Gonad stage	Color of the ovary	Ovarian index	Oocyte diameter (lm)	Molt stage
Control (n=25)	Previtellogenic	White	0.22±0.01	31.80±1.95	Intermolt
Concurrent control (n=25)	Previtellogenic	White	0.23±0.02	33.92±3.43	Intermolt (100)
PDC			(+7.43)	(+6.67)	
P Value			NS	NS	
MF injected (n=25)	Vitellogenic Stage I (n=8)	Light Yellow	0.41±0.01	55.30±2.21	Premolt (24)
PDC			(+85.27)	(+73.89)	
P value			P<0.001	P<0.001	
	Vitellogenic Stage II (n=4)	Dark Yellow	0.52±0.02	62.10±2.84	Intermolt (16)
Premolt (8)					
PDC			(+135.29)	(+95.28)	
P value			P<0.001	P<0.001	
	Vitellogenic Stage III (n=10)	Brown	1.28±0.08	73.90±2.02	Intermolt (20)
Premolt (20)					
PDC			(+478.65)	(+132.39)	
P value			P<0.001	P<0.001	
	Vitellogenic Stage IV (n=10)	Brown	1.31±0.03	83.40±1.89	Molted (12)
PDC			(+491.73)	(+162.26)	
P value			P<0.001	P<0.001	

Values are mean±S.D.

Values in parentheses are percentage change from control prawns.

Table 2: Effect of injection of methyl farnesoate on molting and reproduction in male prawn, *Penaeus monodon*

	Testicular Index	Testicular Follicle Diamenter (µm)	Molt stage
Control (N=25)	0.686±0.0302	37.44±2.73	Intermolt
Concurrent Control (N=25)	0.7292±0.0454	38.96±2.65	Intermolt (100)
PDC	(+6.29)	(+4.05)	
MF - Injected* (N=25)	2.169±0.0887	94.90±3.17	Premolt (80)
			Molted (20)
PDC	(+ 197)	(+ 153)	
P Value	P<0.001	P<0.001	

Values are Mean±SD

Values in Palentheses are% change over Controls.

Among MF Injected prawns (N=25), five prawns molted before completion of the experiment (22 days)

the control values. The ovarian index (0.52±0.02) and mean oocyte diameter (62.10±2.85) of vitellogenic stage-II prawns were significantly increased when compared to control prawns. Similarly the ovarian index (1.28±0.08) and oocyte diameter (73.90±2.84) of vitellogenic stage-III prawns also shown a significant increment compared to control values. The ovarian index (1.31±0.03) and oocyte diameter (83.4±1.89) of vitellogenic stage-IV Prawns also shown a significant increment compared to control values (Table 1).

In male prawns, administration of MF resulted in a significant (P<0.001) increase in testicular index (+197) and mean testicular follicle diameter (+153) when compared with the initial control prawns. No significant change was observed in the concurrent control prawns when compared with the initial control prawns (Table 2).

Regulation of molting in crustaceans involves the steroid molting hormones, the ecdysteroids, which are known to be secreted from Y-Organ. Y-Organs, the source of molting hormone are paired ectodermal derivatives lie just beneath the epidermis. Much evidence shows that they play a positive role in the control of molting. Even though there is a large body of information available for the presence of MF in crustaceans, relatively few data have been provided concerning the physiological roles of MF. The possible roles of MF in regulating crustacean reproduction appear to have received more attention than any other physiological processes [3, 11, 12, 24]. The evidence for the role of MF in the regulation of crustacean molting is also increasing [25, 26].

The evidence for a role of MF in the regulation of molting and reproduction in crustaceans is also

increasing. The functions of MF in crustaceans appear to be multifold but playing a vital role on the promotion of molt cycle in prawns, crabs etc [27]. Chang [1] reported that MF added to culture water containing first stage larval lobsters increased their ecdysteroid concentrations on the hemolymph. MF promotes the ovariam development and maturation in crayfish *Procambarus clarkii* [3] and in crab *Oziotelphusa senex senex* [11]. By using radiolabelled techniques Wainwright *et al.* [5] reported that MO's are responsible for the secretion of MF in edible crab *Cancer pagurus*. Laufer *et al.* [3] showed that in *P. clarkii* the exogenous MF can stimulate and enhance ovarian maturation by stimulating a great number of oocytes to mature. Landau *et al.* [28] reported the synthesis of MF in the mandibular organ of the crayfish *Procambarus clarkii* and its involvement in control of several functions. The present study provides evidence that MO has a role in regulating molting and reproduction processes in the penaeid prawn *Penaeus monodon*, thorough the secretion of MF. But whether MF is directly involved in the regulation of molting or acts by stimulating the ecdysteroid production by the Y-Organs in *P. monodon* is not yet established. Thus, the foregoing evidence supports the hypothesis that MF is a key regulatory factor involved in growth and reproductive development on crustaceans. The studies and to quantify the circulating levels of MF and ecdysteroids during molting and reproduction will through more light on the endocrine regulation of these processes in penaeid prawn *Penaeus monodon.*

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