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Standardization of rearing techniques and biology of predatory mite, *Amblyseius largoenis* (Muma) on red palm mite, *Raoiella indica* Hirst and protocol for its field release

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ABSTRACT: The Red Palm Mite (RPM), *Raoiella indica* Hirst (Acari; Tenuipalpidae) has emerged as a serious pest of palms, especially coconut in Trinidad and Tobago, West Indies. Out of the natural enemy fauna recorded, the predatory mite, *Amblyseius largoenis* (Muma) was found as a potential and most widely occurring predator of red palm mite. Studies were conducted to standardize its rearing technique. Among three types of techniques tested, culturing on the potted coconut seedlings and container method were found to be better option for the mass culturing of *A. largoenis* over arena method. Besides that the population growth parameters of both prey (RPM) and predator were also studied and discussed along with feeding potential of *A. largoenis*. Further, this study will acts as a base line for integrated management practices of *R. indica* as well as for establishment of laboratory for mass culturing of mites.

Keywords: *Raoiella*, Red palm mite, Trinidad and Tobago, predator, mass rearing, *Amblyseius largoenis*

INTRODUCTION

Coconut is an important tropical perennial crop which is an economically important predominant crop in Asia, Pacific, African countries. It is a crop of small holders in many countries and has become an integral and inseparable part of a culture, tradition and religion. In Trinidad and Tobago, coconut was one of the major export tree crops which occupied approximately 4,000 ha in small and large holdings and contributing its share in the country's economy. The coconut crop being an integral part of beaches in Caribbean region, found a potential source of income in the tourism industry. After the entry of Red Palm Mite (RPM), *Raoiella indica* into the Caribbean region in 2004 (Fletchmann and Etienne, 2004) and Trinidad in 2006, it is considered as one of the factors hampering coconut production Further, impact of this invasive alien pest has been reflecting in agriculture, cultural heritage, ecotourism, biodiversity and the industrial sectors of Trinidad and Tobago. Damage to coconuts results in 70 per cent yield reduction and possibly job losses leading to a major socio-economic problem for some of the islands (Roda *et al.,* 2012).

The RPM, *Raoiella indica* Hirst (Acari: Tenuipalpidae) also known as the coconut mite, coconut red mite, red date palm mite, leaflet false spider mite, frond crimson mite and scarlet mite was described and figured by Hirst (1924) from coconut leaves in Coimbatore, India. The biology of *R. indica* maintained on leaf arena has been studied by a number of researchers in other countries. Previous studies showed that the population growth parameters of *R. indica* such as developmental rate, longevity and fecundity depend on the environmental conditions such as temperature, relative humidity and host plants (Cocco and Hoy, 2009; Flores-Galano *et al.,* 2010 and Gonzalez-Reyes and Ramos, 2010). Although RPM management has relied on chemical compounds, there is some knowledge about its natural enemies, mainly in the eastern hemisphere (Pena *et al.,* 2006). Recent information has shown that *Amblyseius largoensis* (Muma) is very common on coconut palms and thus is the primary predator associated with RPM (Carrillo *et al.,* 2012 and Domingos *et al.,* 2013) suggesting that it can play a role in controlling RPM (Carrillo *et al.,* 2010). A classical biological control program can be initiated by identifying potentially effective predatory mites from areas where the RPM is endemic (Hoy *et al.,* 2006).

Hence an attempt has been made on the mass culturing of prey and predators in the laboratory to know the feasibility among different types and details of biology as well as life stages of respective species. The knowledge relating to feeding efficiency of predatory species is also essential to make use of the particular predator for the pest suppression under field conditions. Hence laboratory studies were conducted to determine the life cycle of RPM and its natural enemy; and feeding potential of natural enemy.

MATERIALS AND METHODS

Establishment of coconut seedlings in greenhouse

Six months old coconut seedlings were collected from the field, washed with running water and dried with a cotton cloth. Seedlings were planted in a pot having pot mixture. Adequate watering was given regularly and seedlings were placed in the green house with adequate natural light.

Culturing of stock colony of RPM on coconut seedlings in the greenhouse

Five coconut seedlings were cleaned carefully with a brush to remove all other arthropods. Yellow tagging tape covered with petroleum jelly was tied around the base of the palms to exclude any crawling arthropod. Three hundred RPM, females collected from the field samples were placed on a rectangle piece of coconut leaf (15cm x 2.5cm). The rectangle leaf bits with 300 RPM females were stapled to the lower leaf of the potted coconut seedlings at one leaf bit/plant to facilitate RPM infestation of the coconut seedling (Fig. 1 and 2). The potted coconut seedlings were covered with nylon net fixed with the help of PVC pipes (Fig. 3). Plants were maintained undisturbed for 45 days to allow the multiplication of RPM on the seedling. The procedure was repeated every week to infest a new batch of potted plants. Different stages of RPM were collected from these seedlings and used to inoculate new seedlings for laboratory multiplication of RPM to feed *A. largoensis* and other studies in the laboratory.

Culturing of RPM, *Raoiella indica* in the laboratory as prey

Various trials were pursued for rearing of RPM in the laboratory. The materials required for making test arenas, the dimensions of the leaf bit and the container to be used, the types of sponge, black paper, as well as the tips for maintaining the life span of leaf bit *etc.,* were studied thoroughly and the following types were found suitable for culturing of mites in the laboratory.

Arrangement of Arena

Samples of RPM infested coconut leaf and fresh leaves were collected from field. Fresh leaves were washed with running tap water and then cotton dried. Different containers *i.e.,* the metal tray (32.5cm x 26.5cm x 4.5cm) (Type-1), plastic tray (18cm x 12cm x 7cm) (Type-2), and petri dishes (size 4cm, 8cm diameter) (Type-3a), were sanitized and dried. The sponge was cut into pieces of similar size to the respective container and pre-soaked in water for 20 minutes. Sponge pieces were placed according to the size of container *i.e.*, metal tray (27cm x 20cm x 3.5cm), plastic tray (16cm x 10cm x 3.5cm) and petri dishes (6cm x 6cm x 2cm) to fit exactly into the container. Fresh leaves collected from the field were measured with a ruler and cut into required sizes (Fig. 4) (24cm x 2.5cm – Type 1, 8cm x 2.5c – Type 2, 8cm x 2.5cm – Type 3a and 4cm x 2.5cm – Type 3b). The cut leaf bits were placed on the respective size sponges and fastened with insect pins. The required number of RPM females (50) was transferred to leaf arenas from field sample or stock colonies reared on potted coconut plants in green house with the help of a pig hair brush. Black construction paper was placed around each arena to prevent mite escape. The containers were filled with water to the level of the sponge. Arenas were prepared at periodical intervals to make continuous availability of prey mites. These arenas were maintained in laboratory at $27^{o} \pm 1^{o}$ C temperature, 70% relative humidity and 12:12 photoperiod. (Table 1 and Fig.4)

Biology of RPM

RPM life cycle was studied under laboratory conditions by releasing five laying females on arena Type 3a and 3b (Fig 4). Eggs of same day laying were pooled on to a separate arena of same Type and monitored till adult emergence. Observations on date of egg laying, date of eclosion, date of first moult, date of second moult and third moult and duration of each life stage, longevity of adult, average egg laying, fecundity *etc.,* were recorded.

Mass Culturing of *A. largoensis*, a predator of RPM

The eggs of *A. largoensis* were obtained by keeping the female adults in individual glass vials (3"x1"). As soon as the eggs hatched, larvae were individually transferred to experimental arenas. Arena Type 3b was used for studying the life cycle of *A. largoensis.* The small leaf bit containing RPM eggs was placed over the arena having *A. largoensis* to replenish the diet (RPM) daily. Arenas were replaced when leaf was dried. Observations were recorded daily from arenas to obtain details of life stages of *A. largoensis.*

Mass culturing of the natural enemy, *A. largoensis* in the laboratory was attempted in three methods:

Culturing on potted coconut seedlings

RPM colonized coconut potted seedlings were inoculated with 25 pairs of *A. largoensis* adults on each seedling by placing a glass vial containing *A. largoensis* adults at the collar region of seedling. Seedlings were protected with caging and maintained with adequate water and sunlight (Fig. 3). *A. largoensis* population on the seedling was monitored at fortnightly intervals by

Table 1. Types of containers and arena sizes used for mass rearing of predatory mite

Type	Details	Container size	Sponge size	Size of Leaf bit	Purpose
Type 1	Metal tray	32.5cm x 26.5cm x 4.5cm	27.0cm x 20.0cm x 3.5cm	24.0cm x 2.5cm	Mass culturing of RPM
Type 2	Plastic tray	18.0cm x 12.0cm x 7.0cm	16.0cm x 10.0cm x 3.5cm	8.0cm x 2.5cm	Mass culturing of RPM/arenas for laying females of RPM
Type 3a	Petri dish	8.0cm diameter	6.0cm x 6.0cm x 2.0cm	8.0cm x 2.5cm	Biology Studies of RPM/ natural enemy, *Amblyseius largoensis*
Type 3b	Petri dish	4.0cm diameter	3.0cm x 3.0cm x 2.0cm	4.0cm x 2.5cm	Biology Studies of RPM/ natural enemy, *Amblyseius largoensis*

observing 5 randomly selected areas on leaves. The same procedure was followed to inoculate the other seedlings. After 30 days *A. largoensis* can be harvested for release in the fields.

Culturing on RPM infested coconut leaf bits in a plastic container

Coconut leaf bits of 30cm length were cut from the field collected RPM infested leaf samples or potted coconut seedlings. Leaf bits were cleaned to ensure that they were free from other arthropods. Ten leaf bits were placed in each plastic container (2' x 1 ' x 2 ') and maintained under laboratory conditions. Leaf bits were monitored at 3-day intervals and dried leaf bits were replaced with fresh leaf bits containing RPM. The increase in the number of *A. largoensis* was recorded from 60 to 100 in 7 days in a single container. The containers can be maintained for allowing 30 days for the multiplication of *A. largoensis*. After building the population of *A. largoensis* in the container, they can be used for release in the field.

Culturing in the arena – Type 3

Culturing of *A. largoensis* on leaf arenas was also tried with arena of Type 3b (Fig. 4). In spite of series of approaches, the culture could not be established on the arena. Even though monitoring of samples was found to be critical and difficult, culturing on the potted coconut seedlings and containers method were found to be better options for the mass culturing of *A. largoensis* over arena method. Monitoring of *A. largoensis* culture on seedling revealed suppression of RPM population but *A. largoensis* population could not be seen from sampling leaf area.

RESULTS AND DISCUSSION

Mass vulturing of RPM on Arena

For mass culturing of RPM, arena type 1 was found to be suitable when compared to other types in terms of all aspects. Fifty pairs of female and male RPM were placed on each arena of Type 1 for egg laying and further multiplication. The arenas were replaced with fresh leaf bits when the leaf dried. From each arena with 50 females, 120 eggs were obtained in 10 days. The required RPM were collected from these arenas for making new arenas. All the arenas were monitored and maintained for getting further cultures.

Biology of RPM

While rearing methods were being tried the biology of the prey i.e., RPM was recorded which enables timely handling of prey and its multiplication. Different life stages of RPM were described and durations were recorded

Egg: Eggs are red in colour, smooth and laid in groups on the arena. The number of eggs in a group or colony varied from 11 to 52. Each female laid on an average, 6.67 eggs with a minimum of 3 to maximum 11 numbers in a life period of 17 to 21 days under laboratory conditions. Pre-oviposition period ranged from 5 to 10 days with an average period of 8.3 days and oviposition period varied from 4 to 9 days averaging 6 days. Incubation period was recorded from 4 to 9 days with an average period of 5.87 days.

Larva: Larval stage is having three pairs of legs. The larval, protonymphal and deutonymphal periods ranged

from 2 to 11, 2 to 6 and 2 to 9 days, respectively. The average duration of larval, protonymphal and deutonymphal stages was 6.48, 4.39 and 6.04 days respectively.

Life cycle: The total duration of immature stages varied from 6 to 26 days with an average of 16.91 days. The life cycle of RPM was completed in 18 to 33 days with an average of 22.78 days. The female adult laid on an average one egg/day and it varied from 1-2 eggs/day. Female adult lived up to 17 to 21 days whereas male lived up to 9 to 16 days. Mating duration was observed for about 2 to 4 days.

Biology of *A. largoensis*

Egg: Eggs are in cream colour and laid singly. Each adult laid 1 or 2 eggs/day. Eggs were hatched after a period of 3.52 days on an average; which varied from minimum one day to maximum seven days.

Larva: Larvae were hyaline in colour with three pairs of legs and developed into protonymph with 4 pairs of legs in 3.38 days on an average. Larval period was ranged from 2 to 6 days. Protonymph moulted into deutonymphal stage which was also having 4 pairs of legs and bigger than a protonymph. The protonymphal stage was completed in 2.2 days on an average. The deutonymph took 4.9 days to become adult which was ranged from 5 to 11 days. Protonymph is hyaline in colour and red coloured intestinal contents could be seen distinctly from deutonymphal stage which indicates feeding on RPM. Duration of immature stages was occupied on an average 10.46 days. Deutonymph developed into adult with 4 pairs of legs and little bigger than deutonymph in size.

Life Cycle: Total life cycle from egg to adult was completed on an average of 14.00 days. Minimum period of life cycle was 10 days whereas that of maximum was 17 days. Adult *A. largoensis* lived for a maximum of 22 days under laboratory conditions.

Feeding potential of *A. largoensis*

The potency of a predator is derived based on its feeding potential which will result in pest reduction. Hence these studies were attempted in the laboratory. *A. largoensis* adults were starved for 24 hours by isolating individually into 20 glass vials (8.5 cm x 2.3 cm). Leaf bit containing 40 RPM eggs was introduced into the vial and the number of eggs consumed in 24 hours was recorded. Eggs were provided continuously until *A. largoensis* died. Data were recorded daily and tabulated for determining the feeding potentiality of *A. largoensis*. From the studies on feeding potentiality, it was observed that field collected *A. largoensis* when fed 24hrs after starvation lived up to 3-10days. Feeding potential of *A. largoensis* was varied from 6.14 to 24.38 RPM eggs in 24hrs. On an average 15.53 number of RPM eggs were consumed by single *A. largoensis* female within 24hrs.

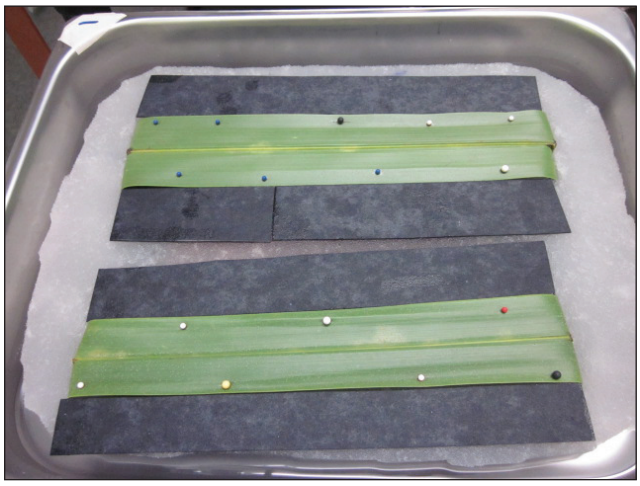
Suggested Protocols for field release of *A. largoensis*

Based on the information derived from above experiments, the final use of predatory mite in RPM infested gardens was suggested to implement practically for the reduction of the RPM population.

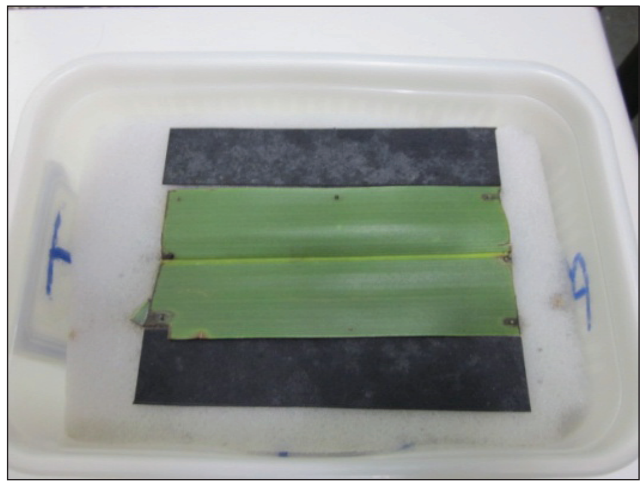
- Collect leaf bits (6cm) with 100 pairs of *A. largoensis* from seedling or plastic container
- Clip the leaf bits to the leaves of coconut palm in opposite directions in the RPM infested field at the rate of 2 leaf bits per whorl of leaves.
- Or carry the leaf bits in zipper locked polythene bags and place in between the fronds
- Open the zipper lock to facilitate the *A. largoensis* to move on to palm
- Evening hours would be congenial for release
- Periodical releases can be made at 15 to 20 days intervals

Limited studies have been performed on the biology of *R. indica* in various countries. However, it was tricky to compare the current results with the others as the experimental conditions (temperature, humidity and host plant) were diverse. Moutia (1958) found that *R. indica* required 18−26 days to develop from eggs to adult at 24.2°C on coconut leaves, while Hoy *et al.* (2006) reported the total development of immature stages was in 23−28 days (for females) and 20−22 (for males) where as present study reported that total duration of immature stages varied from 6 to 26 days with an average of 16.91 days. Nusantara *et al.* (2017) reported that development times was 26.07–28.43 days for females and 23.57−26.89 days for males, showing that the development time of *R. indica* was longer than the present study which revealed that female adult lived up to 17 to 21 days whereas male lived up to 9 to 16 days. Longer immature developmental times of *R. indica* was reported by Galano-Flores *et al.* (2010) (29.72 for females and 32.7 for males) at 25.4°C on areca nut leaves. The female longevity on this study was 17 to 21 days, showing that current study was in line with Vasquez *et al.* (2014) at 29°C (21.5 days). However, the current study was shorter than the report from Nageschachandra and Channabasavanna (1984) which mentioned that the mite longevity was 48.6−50.9 days on coconut leaves.

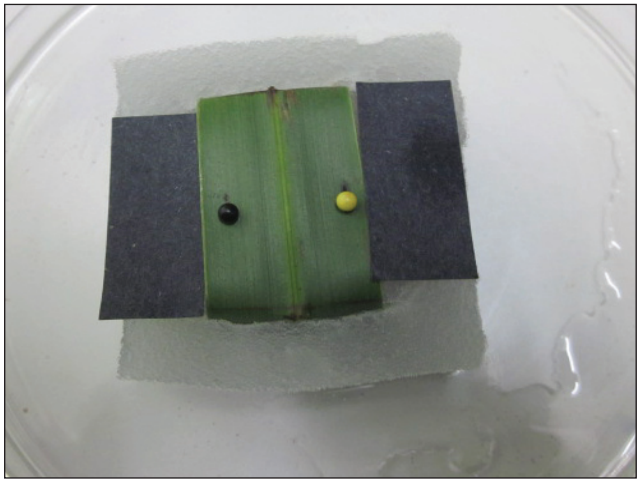
Finally, the results of this study will be important for the management of *R. indica,* a potential pest of coconut in Trinidad and Tobago by providing a better understanding



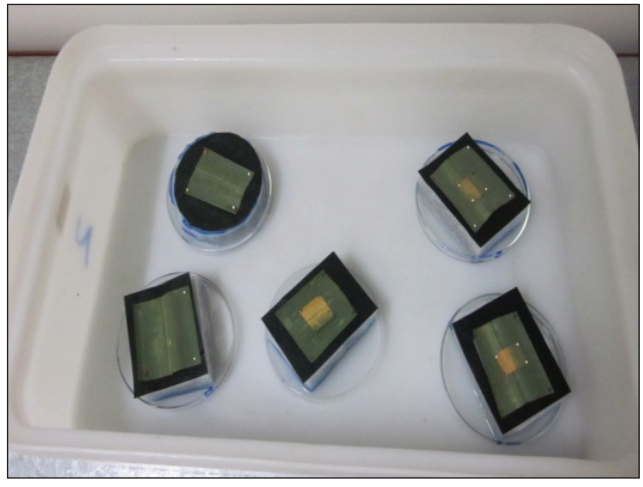
Type 1 - Arena in Metal Tray



Type 2 - Arena in Plastic Tray



Type 3a - Arena in Petri Dish



Type 3b - Arena in Petri Dish

Fig. 1. Different Types of Arena

Fig. 2. Inoculation of RPM

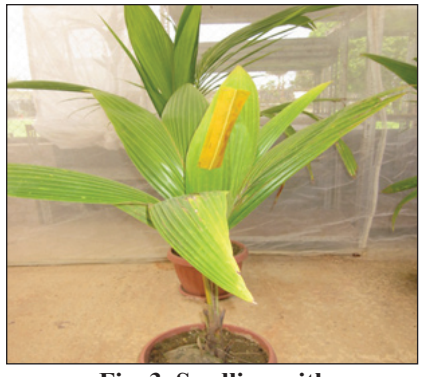


Fig. 3. Seedling with Stapled Leaf Bit

Fig. 4. Caging of Coconut Seedlings

of its biology on coconut. Besides that, studies on the natural enemy of RPM, *A. largoensis* proved that an important mortality factor and a key biological control agent in integrated pest management practices targeting RPM. Previous studies also indicated that *A. largoensis* actively responding in the invasion by RPM and has the potential to be used in biological control and IPM programmes. Moreover, the search on the additional natural enemies, including fungal pathogens should be focussed to suppress the population.

Further investigations are required to determine

best method of approach. The potentiality of natural enemy also needs to be evaluated under field conditions with repetitive and long term field studies like rate of release, number of releases, type of release (Inoculative or Inundative) are necessary to evaluate a potential bio agent. To conduct the above field studies, the continuity of the culturing of mites in the laboratory is necessary. The scale of bio agent production in a laboratory with existing facilities and economics of mass production are yet to be worked out. Ultimately to manage the pests which are invasive alien species, like RPM, Woolly aphid, Cottony cushion scale, Spiralling whitefly, Rugose spiralling whitefly etc., the best option would be to adopt classical biological control in addition to eco friendly IPM strategy instead of opting chemical pesticides.

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