A simple protocol for rearing a native predatory mite *Neoseiulus indicus*

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The indigenous phytoseiid predatory mite *Neoseiulus indicus* (Narayanan and Kaur) was recorded by several Indian researchers as a predator of different species of phytophagous mites including *Oligonychus indicus*, *Tetranychus urticae*, *T. neocaledonicus*, *T. ludeni*, *T. macfarleni*, *Polyphagotarsonemus latus* and the astigmatid mite *Tyrophagus putrescentiae.* For the first time in the country, a simple protocol has been developed for mass rearing of *N. indicus* in closed units on the astigmatid mite *T. putrescentiae*, which in turn could be maintained on a wheat bran medium. *Neoseiulus indicus* has been successfully and continuously mass reared for more than four years at the ICAR-NBAIR, Bengaluru, India. A simple release methodology has also been developed for the mass reared predatory mites. This rearing protocol can be adopted by commercial units and also by farmers/polyhouse growers as an on-farm production system.

Keywords: Astigmatid mite, mass rearing, phytoseiid predatory mite, simple rearing protocol.

TETRANYCHID mites cause serious damage to several agricultural and greenhouse crops, and the indiscriminate use of broad-spectrum acaricides has led to the development of resistance in pest mites, viz. *Tetranychus* spp. In several countries, phytoseiid mites have been commercially produced and utilized as bioagents primarily against *Tetranychus urticae* Koch and also against other species of mites, thrips and whiteflies[1]. The promising predatory mites which are being globally used as bioagents are *Phytoseiulus persimilis* Athias-Henriot, *Neoseiulus cucumeris* (Oudemans), *Neoseiulus barkeri* Hughes, *Neoseiulus californicus* (McGregor), *Neoseiulus fallacis* (German), *Iphiseius degenerans* (Berlese), *Galendromus occidentalis* (Nesbitt) and *Amblyseius swirskii* Athias-Henriot[2–6]. Predatory mites have been mass produced by several researchers[7,8]. In India, though about 217 species of phytoseiids have been recorded[9,10]; *Neoseiulus longispinosus* (Evans) is reported as the most potential obligate predator of many tetranychid mites[11], attacking a wide range of fruit crops, field crops and ornamentals. A protocol was developed in India to rear *N. longispinosus* on *T. urticae* maintained on pole bean or French bean plants in glass/polycarbonate houses[12–14]. Different host plants and pest mite species have also been used for mass rearing *N. longispinosus*[15,16].

The predatory mite *Neoseiulus indicus* (Narayanan and Kaur) (Mesostigmata: Phytoseiidae) was originally described in India based on material collected from *Phthorimaea operculella* (Zeller)-infested potatoes, feeding on *Tyrophagus putrescentiae* (Schrank)[17]. It was later recorded from wheat and maize in Punjab; maize in West Bengal; sorghum in Gujarat, and okra and brinjal in Lucknow, Uttar Pradesh[18–24]. It was recorded as a predator on all stages of phytophagous mites on maize in Punjab[22] and on the eggs of *Oligonychus indicus* (Hirst) infesting maize in West Bengal[25]. In his review on oriental Phytoseiidae, Gupta[26] has mentioned *N. indicus* to be recorded only from India. Besides being recorded as a predator of *T. urticae*[27], *N. indicus* has also been recorded as a predator of *Tetranychus neocaledonicus* Andre[28], *Tetranychus ludeni* Zacher infesting citrus[29], broad mite *Polyphagotarsonemus latus* (Banks) infesting mulberry[30] and pumpkin mite *Tetranychus macfarleni* (Baker and Pritchard)[31]. However, no systematic attempt has been made to mass rear and release *N. indicus* as a biocontrol agent to manage different species of phytophagous mites. Studies have indicated that astigmatid mites like *Tyrophagous putrescentiae* can be used as prey for rearing predatory mites[32,33]. Besides, *N. indicus* was observed to feed on *T. putrescentiae* by Narayan and Kaur[17], when it was first described, indicating that *T. putrescentiae* could work as an alternate prey for rearing *N. indicus*. This communication describes a simple rearing protocol developed for mass rearing *N. indicus* on the astigmatid mite *T. putrescentiae.*

Tyrophagus putrescentiae (Astigmata: Acaridae), a prey mite, was used for rearing the predatory mite *N. indicus* in the laboratory at 26° ± 2°C and 60 ± 10 relative humidity (RH). A rectangular plastic box (140 × 95 cm and 50 cm height) was used as the rearing container (Figure 1 *a*). The lid was fitted with a wire mesh (100 mesh size) for ventilation. A rectangular piece of filter paper placed at the base of the container was strewn with wheat bran @ 50 g per container and 0.5 cm^3 of dry yeast. A cotton swab soaked in water was stuck to the side of the container. This fresh box was charged with culture of storage mites (approx 1 cm^3). The container was then covered with a piece of washed (to remove starch) black poplin cloth and then tightly closed with the lid (Figure 1 *b*). The rearing containers were arranged in a plastic tray and the whole set-up was placed in a tray of water to avoid escape of the mites (Figure 1 *c*). On alternate days, fresh wheat bran and yeast were provided to the containers and swabs moistened with water every day.

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Figure 1. Rearing units for *Tyrophagus putrescentiae*. ***a***, The rearing container with a mesh-fitted lid for ventilation. ***b***, Inner contents of the container with a black cloth lining for the lid. ***c***, The whole set-up of the rearing containers in a tray placed inside another tray of water.

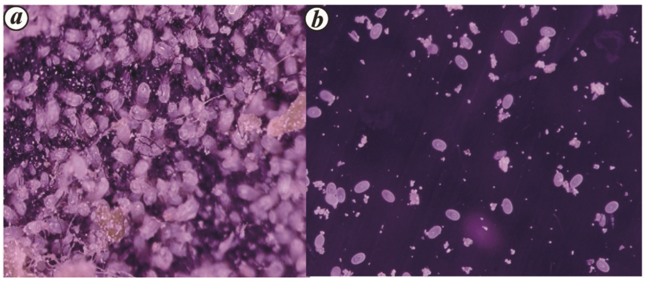

Figure 2. *T. putrescentiae* in culture: ***a***, nymphs and adults; ***b***, eggs.

Figure 3. Rearing units for *Neoseiulus indicus*. ***a***, Rearing containers. ***b***, Inner contents of the rearing container. ***c***, The set-up of rearing containers placed inside a tray of water. ***d***, The rearing containers stacked on racks.

The predatory mite *N. indicus* was reared in the laboratory at 26° ± 2°C and 60 ± 10 RH using round plastic containers (160 cm diameter and 75 cm height) as rearing units. A circular hole (2 cm diameter) was punched exactly at the centre of the lid. A piece of wire mesh (100 mesh size) was fixed on the lid to cover the hole on the outer side (for ventilation) and a piece of nylon mesh was fixed on the inner side of the lid exactly opposing the wire mesh to prevent escape of the predatory mites. A circular piece of filter paper (155 cm diameter) was placed at the base of the container and 20 g of vermiculite poured on the paper with 1 cm^3 yeast granules and pollen (castor or maize) was sprinkled with a brush. Castor leaf with its stalk covered using moist cotton, and further covered with a silver foil was placed at the base of the container. A cotton swab soaked in water was stuck to the wall of the container (to be moistened daily). A layer of cotton was placed inside the container. In order to initiate the culture, eggs/nymphs/adults of *N. indicus* from an existing culture were incorporated into the container. Around 1–2 cm^3 of storage mites (Figure 2 *a* and *b*) (from the storage mite rearing containers) was added as feed for the predatory mites. Eggs of storage mites (Figure 2 *b*) can be stored in a refrigerator for up to seven days and provided as feed for *N. indicus*. The container was covered with a piece of black poplin cloth, on which a layer of cotton was placed, followed by a piece of black cloth and one more layer of cotton, and then the container was covered with the lid (Figure 3 *a* and *b*). The cotton strands and threads of the black cloth act as oviposition substrates (Figure 4 *a*). Also, the double layer of black cloth can help prevent the escape of nymphs and adults of predatory mites to a large extent. The containers were inverted and stacked in trays, which were further placed inside a larger tray of water. This helps prevent the escape of predatory mites (Figure 3 *c*). The trays were stacked on racks (Figure 3 *d*). The nymphs and adults of *N. indicus* fed actively on the eggs and nymphal stages of storage mites and multiplied (Figure 4 *b–d*). On alternate days, storage mites, 0.5 cm^3 yeast and pollen were provided to the predatory mite containers. Eggs of *N. indicus* laid on the cotton strands and threads of black cloth were collected on alternate days and added to a fresh container to continue the predatory mite culture. Fresh leaves were provided according to need. Under a stereozoom microscope, large populations of predatory mites could be observed between the two pieces of black cloth (covering the rearing container) amidst the cotton strands.

From one container, around 800–1000 mites could be harvested per week. One contractual worker can handle around 20 storage mite containers and 20 predatory mite containers per day and hence in a week, approximately

20,000 mites can be harvested from a small rearing facility by engaging just one contractual worker. Minimum investment is required on the following: room space for production to hold 10 racks and other items, viz. plastic trays, plastic boxes, cloth covers, vermiculite, yeast, wheat germ and pollen, besides nucleus cultures of host and predatory mites. Certain precautions have to be followed under this rearing protocol. (i) Since *T. putrescentiae* can cause allergy, workers have to use masks and gloves while handling the cultures. (ii) In order to avoid cecidomyiid flies as contaminant in the culture, castor leaves have to be cleaned well and shade dried before putting them into the rearing containers. (iii) Care has to be taken to see that there is sufficient water in the outer trays (in which the rearing units are placed).

Unlike chemicals, parasitoids and predators can seek out prey and are not limited to the area to which they are applied[34]. Exotic phytoseiid mites like *P. persimilis* have also been imported and evaluated in India; however, their establishment has not been significant. Indigenous phytoseiid mites have been utilized widely in biocontrol programmes due to their superior biological parameters, which include high fecundity, good searching ability, dispersal rate, adaptability to different ecological niches and a high degree of prey specificity. However, in recent years there has been a move from specialist to generalist predators, which besides being abundantly available can persist on alternative prey, viz. phytophagous mites, storage mites and even on pollen in the absence of the target pest[35,36]. Plant-based rearing systems comprising greenhouses occupying large areas of land with facilities like heating/cooling/lighting are essential for rearing specialist predatory mites. Hence, globally there have been attempts to move from the plant-based rearing system to plant-less, factitious, host-based rearing system in order to avoid the costs involved in maintaining the greenhouses, and also considering the labour costs and the yield of predatory mites per unit area of production. There are several studies focusing on rearing and evaluating the indigenous obligate predatory mite *N. longispinosus*[12,14]. *Neoseiulus longispinosus* being an obligate predator is reared on *T. urticae* infested plants in green houses. Though *N. indicus* has been recorded as a predator of different phytophagous mite species, there has been no attempt to rear and release this predatory mite as a biocontrol agent on different species of pest mites infesting different crops. *Neoseiulus cucumeris* has been reared successfully on *T. putrescentiae*. El-Atta and Osman[37] developed a rearing method for *T. putrescentiae*, wherein they reported that egg-laying could be increased using yeast in the medium. Breeder piles of the phytoseiid predatory mite *N. cucumeris* along with an admixture of bran and *T. putrescentiae* mould mites have been utilized effectively in biocontrol programmes. In breeder piles, the fungus which grew on bran served as feed for *T. putrescentiae*, which in turn supported as prey for *N. cucumeris*. The predatory mites were loaded in carrying tubes which contained bran flakes, vermiculite and *T. putrescentiae*, and the contents were sprinkled into the greenhouse crops[5,38]. In the present study, the indigenous predatory mite *N. indicus* has been successfully reared on a factitious prey mite *T. putrescentiae*, which in turn was reared on a wheat bran medium. Since this is a simple protocol not requiring any sophisticated facilities, it can be taken up as an on-farm production system by farmers/ polyhouse growers. The predatory mites from the rearing containers with vermiculite, bran and *T. putrescentiae* can be sprinkled on the pest mite-infested plants. The astigmatid mites which may also get released along with the predatory mites may remain on the plants for some time, serving as alternate prey without causing harm to the plants. The mass-reared *N. indicus* has to be evaluated systematically against different pest mites, especially those phytophagous mites which have earlier been recorded as natural prey of *N. indicus*. This rearing protocol can also be tested for rearing other indigenous generalist phytoseiid mites.

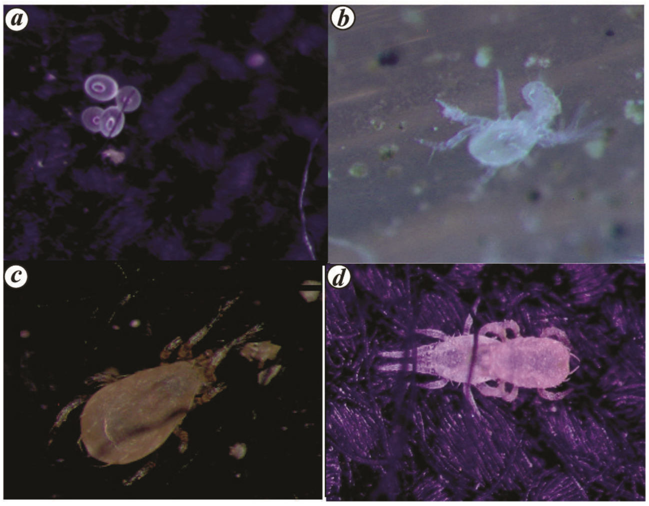

Figure 4. *N. indicus* in culture. ***a***, eggs; ***b*** nymph; ***c***, adult; ***d***, mating pair.

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