Pest Management in Horticultural Ecosystems
Vol. 30, No.2 pp (2024)
DOI Number : 10.5958/0974-4541.2024.00035.5

Efficacy of *Neoseiulus longispinosus* (Acari: Phytoseiidae) in controlling red spider mite, *Tetranychus macfarlanei* Baker & Pritchard on Cucumber: Laboratory and Field Studies

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ABSTRACT: Studies were conducted to evaluate the effectiveness of the predatory mite, *Neoseiulus longispinosus* (Evans) (Acari: Phytoseiidae) against the emerging red spider mite, *Tetranychus macfarlanei* Baker & Pritchard (Acari: Tetranychidae). The study involved laboratory and field trials with different predator–prey ratios of 1:25, 1:50, 1:100, and 1:200. Laboratory trials revealed that hundred per cent reduction of prey mites was achieved at 10 and 12 days after predator release at ratios of 1:25 and 1:50, respectively. Recommending ratios of 1:25 and 1:200 to the farmer were deemed uneconomical. Field trials were conducted with the two other most effective predator–prey ratios, 1:50 and 1:100, and both ratios resulted in a greater than 99% reduction in the prey mite population 20 days after predator release. The study concluded that *N. longispinosus* is an effective and sustainable biocontrol agent for spider mites on cucumber plants. Recommending predator–prey ratios of 1:50 or 1:100 to farmers can lead to significant reductions in pest populations and is cost effective.

Keywords: Tetranychidae, horticulture, predator–prey ratio, biological control

INTRODUCTION

It has long been known that biological control is a reliable and sustainable method of managing pests. Several species of predatory mites are efficient biological control agents against pestiferous mites, insects, and nematodes. In many agro ecosystems, predatory mites are crucial biological control agents for spider mites (Yanar *et al.*, 2019). Phytoseiidae is one of the largest predatory mite families in the order Mesostigmata and includes several potential predatory species. The inoculative release of predatory mites from the family Phytoseiidae is an alternate method of controlling spider mite populations to pesticides. The value of phytoseiid mites for regulating spider mite populations has been well documented (Huffaker *et al.*, 1970; McMurtry *et al.*, 1970).

The predatory mite, *Neoseiulus longispinosus* (Evans) (Acari: Phytoseiidae) has been proven to be the most efficient at controlling spider mites in diverse crops. It has a wide range of distributions and can adapt to hot temperatures within polyhouses in South Indian climates (Mallik *et al.*, 1999). Commercial use of available predatory mites within the context of IPM programs has gained popularity as an economically viable pest management method for greenhouse crops (Vila and Cabello, 2014; Calvo *et al.*, 2015; Van-Lenteren *et al.*, 2018).

Numerous studies have been conducted on the biological control of spider mites in vegetables with the use of predatory mites (Gerson and Weintraub, 2007); However, research specifically focussing on the control of the red spider mite, *Tetranychus macfarlanei* Baker and Pritchard (Acari: Tetranychidae) on crops such as cucumber is scarce. Cucumber (*Cucumis sativus* L.) is an important creeping vine in the Cucurbitaceae family, the fruits of which are used as vegetables and green salads. Cucumber thrives in India's warm climate, and the southern states of Karnataka, Tamil Nadu and Andhra Pradesh are the major producers of cucumber crops. This crop is susceptible to various insect and mite pests, of which *T. macfarlanei* is the more serious pest. Mites colonize the underside of leaves and cause damage by piercing the cells and sucking out the cell contents. In Karnataka, it appears in moderate to severe form on cucumber and other vegetable crops in and around Bengaluru (Latha *et al.*, 2019).

Biological control with the predatory mite, *N. longispinosus* was attempted for the control of the spider mite *T. macfarlanei* and was found to be promising (Anonymous, 2020). The possibility of an interference component between phytoseiid predators and their prey is an essential part of the debate over whether phytoseiids can reduce excessive populations of prey mites (Laing and Osborn, 1974). Predator's functional and numerical responses decline with high numbers of prey (Chant 1961; Mori and Chant (1966); Kuchlein 1967; Mori 1969). The present investigation aimed to determine the potential efficacy of this predator on prey mites. The research on the effectiveness of *N. longispinosus* has demonstrated that it is more promising to employ this predatory mite to manage spider mites on crops like cucumber, pointed gourd, banana, brinjal, okra, rose, carnation, and gerbera in India(Anonymous, 2020). However, additional studies are still needed to determine the efficacy of this predator in controlling spider mites on a variety of vegetable and ornamental crops grown under protected conditions as well as in open fields. Considering this, a study was conducted to determine how well *N. longispinosus* controlled *T. macfarlanei* on cucumber plants through biological control.

MATERIALS AND METHODS

Maintenance of mite cultures

Stock cultures of both the spider mite *T. macfarlanei* and the predatory mite *N.longispinosus* were maintained in the laboratory of the All-India Network Project on Agril. Acarology, UAS, Bengaluru, Gandhi Krishi Vignana Kendra. Excised cucumber leaves were placed abaxial side up on wet foam sheets in 12”×10”×2” plastic trays. In plastic trays, prey mites from the stock culture were released and allowed to develop on excised cucumber leaves. The leaves were changed every 5 to 6 days or whenever they started to dry. To keep the foam sheets moist and the cucumber leaves fresh, the trays were watered every day. Many of these trays were kept throughout, and some of them were utilized for predatory mite multiplication. Cucumber leaves infested with prey mites were plucked from a living plant and piled onto a tray. Subsequently, the predatory mites were released from the stock culture. These predatory mites were allowed to feed and multiply in the same trays.

Laboratory testing of the effective predator-prey ratio for controlling the spider mite *T. Macfarlanei*

The predator-prey ratio required to manage the spider mite, *T. macfarlanei* on cucumber plants was studied in the laboratory by releasing the predatory mite *N. longispinosus* at different predator-prey ratios on spider mite–infested cucumber leaves in plastic trays. In the experiment, there were four replications and five treatments in a completely randomized layout. The treatments included a predator-prey ratio of 1:25, 1:50, 1:100, 1:200, and the control without releasing any predators. Excised cucumber leaf blocks of 2”×2” were placed on the abaxial side up on a saturated foam sheet in plastic trays of 10”×8”×2”. One leaf bit was placed in each tray, and 20 such trays were used for the whole experiment. On each leaf, 200 *T. macfarlanei* mites collected from the stock culture were released at all stages. For each tray in the first set of four trays (treatment 1), 8 gravid females of *N. longispinosus* were tested; for the second set of four trays (treatment 2), 4 predatory mites were tested; for the third set of four trays (treatment 3), 2 predatory mites were tested; for the fourth set of four trays (treatment 4), 1 predatory mite was released to serve as a 1:25, 1:50, 1:100 and 1:200 predator-prey ratio, respectively; and for the fifth set of four trays (treatment 5), no predators were released to serve as the control. The predatory mites were allowed to feed on the prey mites. The cucumber leaf bits were replaced with fresh bits as if they were dry, and the prey and predatory mites on the dried leaf bits were subsequently transferred back to the fresh leaf bits. The trays were watered daily to maintain the foam sheet in a water-saturated condition and to prevent the escape of prey and predatory mites from the arena. Observations of the number of prey and predatory mites on leaf bits were recorded at 2,4, 6, 8, 10, and 12 days after the predator was released. The values were square-root transformed by adding 0.5 and were analysed by one-way ANOVA using R Software (version 4.0.3) (2020-10-10).

Field test of the effective predator–prey ratio for controlling the spider mite *T. macfarlanei* on cucumber plants

The effective predator-prey ratio required to control *T. macfarlanei* on cucumber plants in the open field was evaluated after releasing the predatory mite *N. longispinosus* at two predator-prey ratios on spider mite–infested cucumber plants. Of the four predator-prey ratios tested in the laboratory, two ratios, 1:50 and 1:100, which were most effective at suppressing *T. macfarlanei* in the shortest possible time, were selected for the field study. There were three treatments in the experiment, including the control. The cucumber crop was raised in the field at Zonal Agricultural Research Station, GKVK, Bengaluru, following all the standard agronomic practices. In the experiment, three rows were arranged, each comprising 15 isolated cucumber plants that served as 15 replicates. The cucumber variety used was Suchitra 45, a popular variety among farmers around Bengaluru. The crop was sprayed with the selective insecticides imidacloprid and flubendiamide at their recommended field dosages to control sucking and chewing insects during the crop growth period. Because there was no natural infestation of spider mites on the crop, the cucumber plants were artificially infested with the laboratory-maintained spider mite *T. macfarlanei*@30 mites/leaf at twenty days after the sowing. The spider mites were allowed to establish themselves on the crop. The predatory mites were released at a predator-prey ratio of 1:50 to 15 plants in a row fifteen days after the introduction of the prey mites, and in another row of 15 plants, the predators were released at a 1:100 predatory-prey ratio. The third row was left without releasing the predators to serve as the control. The predatory mites in both treatments were allowed to multiply on the prey mites. Observations of the total number of prey mites on cucumber plants were noted down at five-day intervals until all the prey mites were exhausted or their number reached a minimum threshold. For recording observations, one leaf from each cucumber plant was collected, and in the laboratory, the total number of mites present on the entire leaf was counted under a Zeiss Stemi 2000C stereo zoom microscope. By multiplying by the total number of leaves on each plant, the total number of prey mites per plant was calculated. The per cent reduction in prey mites over the control for both predatory: prey ratios were calculated using the formula suggested by Henderson and Tilton (1955). Following the release of the predatory mites, data was analyzed on the total number of prey mites observed and the percentage reduction over the control at various intervals.

RESULTS AND DISCUSSION

Determination of the effective predator–prey ratio for controlling the spider mite *T. Macfarlanei*

A total of five treatments were used in the laboratory study to determine the effective predator-prey ratio required to control the spider mite *T. macfarlanei* on cucumber plants by releasing the predatory mite *N. longispinosus*. The treatments include four predator-prey ratios of 1:25, 1:50, 1:100, and 1:200 on excised cucumber leaves in plastic trays, with no predators serving as the control treatment. The data recorded on the mean number of prey and predatory mite eggs+active stages on cucumber leaf bits in trays at 2, 4, 6, 8, 10, and 12 days after the predatory mites were released are given in Table 1 and depicted in Fig. 2.

In the scenario where predatory mites were introduced at predator-prey ratios of 1:25, predatory mite populations increased steadily until the eighth day and the peak population was marked on the same day. In treatments with predator–prey ratios of 1:50 and 1:100, there was a continuous increase in the predatory mite population

Table 1. Influence of the predator:prey ratio on the populations of *Tetranychus macfarlanei* (prey mite) and *Neoseiulus longispinosus* (predator) on cucumber leaf discs in the laboratory

Predator: prey ratio	Mean number of *T. macfarlanei* after predator release (eggs + active stages)						Mean number of *N. longispinosus* after predator release (eggs + active stages)					
	DAY 2	DAY 4	DAY 6	DAY 8	DAY 10	DAY 12	DAY 2	DAY 4	DAY 6	DAY 8	DAY 10	DAY 12
1:200	308.50^{a} (17.55)	315.75^{a} (17.75)	246.00^{b} (15.66)	192.75^{b} (13.87)	107.25^{b} (10.34)	60.25^{b} (7.73)	3.75^{c} (2.06)	7.25^{c} (2.78)	9.00^{d} (3.08)	19.50^{d} (4.47)	21.50^{d} (4.68)	32.25^{a} (5.72)
1:100	189.75^{b} (13.78)	164.50^{b} (12.83)	120.00^{c} (10.93)	71.25^{c} (8.38)	33.00^{c} (5.62)	8.00^{c} (2.38)	6.00^{c} (2.53)	10.50^{c} (3.31)	12.75^{c} (3.64)	27.50^{c} (5.29)	28.75^{c} (5.41)	32.00^{a} (5.68)
1:50	155.75^{b} (12.45)	109.50^{c} (10.42)	80.25^{cd} (8.92)	43.00^{cd} (6.51)	4.50d (2.03)	0.00^{c} (0.71)	13.50^{b} (3.73)	24.25^{b} (4.96)	26.75^{b} (5.21)	40.00^{b} (6.36)	37.75^{b} (6.18)	20.25^{b} (4.54)
1:25	139.75^{b} (11.84)	88.50^{c} (9.42)	61.50^{d} (7.86)	18.75^{d} (4.37)	0.00^{d} (0.71)	0.00^{c} (0.71)	21.25^{a} (4.66)	35.50^{a} (6.00)	38.25^{a} (6.22)	61.00^{a} (7.84)	46.75^{a} (6.87)	19.75^{b} (4.46)
Control	336.50^{a} (18.35)	383.75^{a} (19.60)	559.00^{a} (23.64)	647.75^{a} (25.45)	608.00^{a} (24.66)	483.00^{a} (21.98)	0.00^{d} (0.71)	0.00^{d} (0.71)	0.00^{e} (0.71)	0.00^{e} (0.71)	0.00^{e} (0.71)	0.00^{c} (0.71)
F Test	**	**	**	**	**	**	**	**	**	**	**	**
SEM ±	0.45	0.47	0.52	0.52	0.50	0.53	0.12	0.13	0.12	0.11	0.11	0.24
CD (P=0.05)	1.37	1.41	1.56	1.56	1.52	1.60	0.35	0.38	0.37	0.34	0.35	0.72

Values within columns with the same alphabetical superscript are not significantly different (P=0.05).
The figures in parentheses are square root-transformed values.
** indicates significance (@ P=0.01)

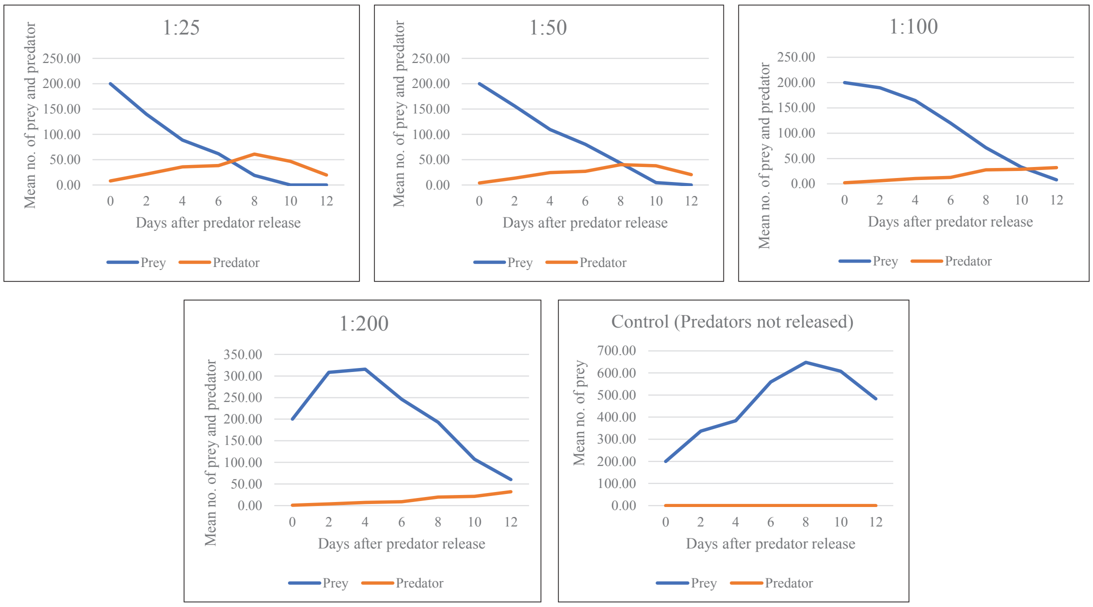

Fig.1. Influence of predator: prey ratio on the population of *Tetranychus macfarlanei* and *Neoseiulus longispinosus* eggs+ active stages on cucumber leaves in the laboratory

until 8 and 12 days respectively. Subsequently, the population of predatory mites became stable without any further increase because of the non-availability of prey mites (Fig. 2).

In treatment 1:200, a persistent decline in the prey mite population was observed from day 2 to day 12 after the initial release of predatory mites. However, effective reduction of the prey mite population was not achieved even beyond day 12.In the control, the prey mites were allowed to multiply without the interference of predatory mites and the prey population consistently rose for the first 8 days, after which it slightly declined. This reduction can be attributed to limited available space for mite establishment and insufficient nutrient support in the leaf substrate for sustaining prey mite culture.

Determination of the effective predator-prey ratio for controlling the cucumber spider mite *T. macfarlanei* in the field

The predatory mite *N. longispinosus* was released at two predator-prey ratios on cucumber plants infested with the spider mite *T. macfarlanei* in the field to determine the effective predator–prey ratio. Considering the feasibility of mass production as well as its effectiveness in suppressing the prey population in the shortest possible time, two predator–prey ratios, 1:50 and 1:100, among the four ratios tested in the laboratory, were taken to the field to test their effectiveness. These two treatments, along with a control (treatment without releasing predatory mites), were tested in the field after releasing *N. longispinosus* on *T. macfarlanei*-infested cucumber plants. The cucumber plants were artificially infested with the prey mite *T. macfarlanei* on a 20-day-old cucumber crop. The predatory mites were released 15 days after infestation with the prey mites at ratios of 1:50 and 1:100 in the first two treatments. The third treatment served as a control in the absence of predatory mites.

Observations of the total number of prey mites per leaf and per plant at 5, 10, 15, and 20 days after the predator release are given in Table 2 and Table 3, respectively.

Table 2: Mean no. of prey mites/leaf on different days after the release of predators

Predator: prey ratio	Before release	5 DAR	Per cent reduction over control (%)	10 DAR	Per cent reduction over control (%)	15 DAR	Per cent reduction over control (%)	20 DAR	Per cent reduction over control (%)
1:100	217.00	307.10	0.42	244.20	47.05	124.10	81.05	6.40	99.30
1:50	212.63	265.10	12.27	182.70	59.57	78.60	87.75	1.80	99.80
Control	232.90	331.00	-	495.00	-	702.80	-	974.80	-

Table 3: Mean no. of prey mites/plant on different days after the release of predators

Predator: prey ratio	Before release	5 DAR	Per cent reduction over control (%)	10 DAR	Per cent reduction over control (%)	15 DAR	Per cent reduction over control (%)	20 DAR	Per cent reduction over control (%)
1:100	7694	10831	11.42	9110	51.48	4753	83.63	258	99.36
1:50	7622	9956	17.81	7274	60.89	3193	88.46	80	99.80
Control	7842	12463	-	19137	-	28479	-	41007	-

There was a continuous decline in the number of prey mites at both predator-prey ratios from 5 to 20 days after the predatory mites were released. A continuous decrease in the number of prey mites per plant was noticed for both predator-prey ratios from 5 to 20 days after the release of predatory mites (Fig. 3).The percentage reductions in the prey population, relative to the control population, exhibited a discernible ascending trend commencing five days post-release, indicating a consistent trend of multiplication with respect to predator-prey ratios. (Fig. 4).Furthermore, at a predator-prey ratio of 1:50 as opposed to 1:100, the rate of decline in the prey mite population was higher. Nonetheless, by 20 days following the introduction of the predatory mites, the percentage decrease in the prey population compared to the control population surpassed 99% for both predator-prey ratios, indicating the effectiveness of both ratios in controlling prey mites within 20 days after the predatory mites were released. In contrast, there was a continuous rise in the population of prey mites in the control.

RESULTS AND DISCUSSION

The efficacy and optimum predator-prey ratio of predatory mites, *N. longispinosus,* for controlling red spider mites, *Oligonychus coffeae* Neither, in infesting tea were studied by Rahman *et al.* (2011). They found that predator-prey ratios of 1:33 and 1:50 were effective in the laboratory, and a 1:25 ratio was found to be effective in the greenhouse. These authors showed that *N. longispinosus* could be used as a successful biocontrol agent for *O. coffeae* in tea through augmentation. In the present study, complete control of the prey mite

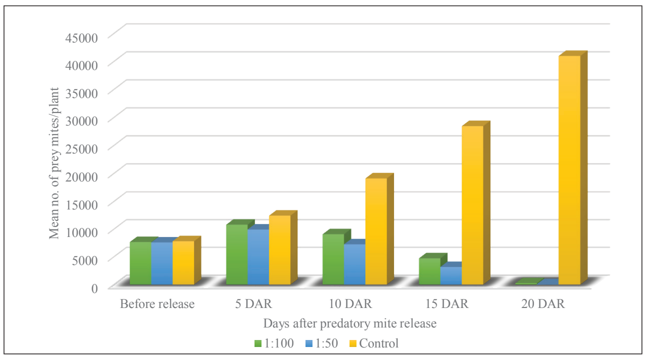

Fig. 2 Effect of predator: prey ratio on the population of *T. macfarlanei* (prey) on cucumber plants in the field

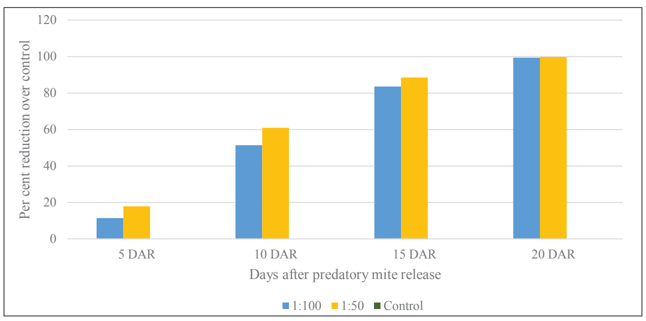

Fig. 3 Effect of predator: prey ratio on the population reduction of *T. macfarlanei* (prey) on cucumber plants in the field

T. marfarlanei was achieved rapidly at 1:25 and 1:50 predator-prey ratios within 10 and 12 days after the predatory mites were released. The small changes in the duration of complete control of spider mites may be attributed to changes in the prey mite species and the plant host. Nevertheless, the consistent rise in the population of predatory mites over the course of 12 days post-release is attributed to prolonged access to prey mites within the 1:200 ratio. The findings by Mondal *et al.* (2020) underscore that the extended longevity of *N. longispinosus* on *T. macfarlanei* renders the prey mite a conducive host for the predator.

The results of the study conducted by Rao *et al.* (2017) indicated that the release of *N. longispinosus* at predator-prey ratios of 1:10, 1:20, and 1:30 could indicate the rapid multiplication of *Tetranychus urticae* Koch, but the plants that received a predator-prey ratio of 1:50 had a greater number of spider mites (40.56/cm^2) with a low pooled mean reduction of 20.62%. In contrast, the results of the present study showed that a predator-prey ratio of 1:50 was the most effective for controlling *T. macfarlanei* on cucumbers in the field. Narrow ratios were not evaluated in this study since they are not cost-effective for farmers to utilize in the field. In the present study, ratios for the field study were determined after completing the laboratory trials.

Lenin and Bhaskar (2019) studied the efficacy of *N. longispinosus*in managing *T. urticae* on cucumbers under laboratory and polyhouse conditions. The results of these studies showed that prey mites were eliminated by the seventh and tenth days at predator-prey ratios of 1:5 and 1:10, respectively, and at wider ratios ranging from 1:20 to 1:100, total elimination of the prey population was not achieved up to ten days after predator release, but there was a significant reduction in the prey population. A study conducted by Rahman and Azariah (2011) also indicated the proficiency of 1:33 and 1:50 ratios under laboratory conditions, while 1:25 was effective in polyhouse conditions. The population of predatory mites in 1:25 after day 8 reached satiation because of the non-availability of prey mites indicating Holling's type II model (Holling, 1959; Ibrahim and Rahman, 1997).However, due to economic considerations, the recommendation of a 1:25 ratio for field conditions was deemed impractical, hence the ratios 1:50 and 1:100, along with a control (treatment without releasing predatory mites), were tested in the field and are proved effective.In a study akin to ours conducted by Ibrahim *et al.* (2006), they observed a 98.70% reduction in *T. urticae* after introducing *Neoseiulus cucumeris* (Oud.) on cucumber over two months. In our current investigation, we achieved a noteworthy 99% reduction in *T. macfarlanei* population in less than a month, underscoring the prowess of *N. longispinosus* as a highly effective predator in the Indian context.

CONCLUSION

The current investigation aims to establish the effectiveness of *N. longispinosus* in managing the spider mite, *T. macfarlanei*. While numerous studies have underscored the potential of this predator, the majority have been conducted in laboratory settings, with a few in polyhouses. This study marks the initial effort to deploy *N. longispinosus* for *T. macfarlanei* control in open field conditions. Despite several limitations in the field experiment, it represents the first successful endeavor in employing *N. longispinosus* to combat this troublesome mite. Future research should concentrate on standardizing commercial aspects such as the packaging and field release of predators. This is a challenging task due to the obligate nature of the predator, and addressing this challenge will be crucial for advancing the application of *N. longispinosus* in pest control.

ACKNOWLEDGEMENT

We would like to extend our sincere thanks to the All-India Network Project on Agricultural Acarology (AINPAA) for providing the necessary logistics and research facilities for conducting this study. Their support has been invaluable in enabling us to carry out our research efficiently and effectively. We also express our gratitude to the staff of AINPAA for their assistance and cooperation during the course of this research.

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MS Received: 27 August 2024
MS Acceptance: 10 November 2024