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RESEARCH ARTICLE
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Co-infection by Root-Knot Nematodes and Spider Mites Increases Susceptibility in Thai Chili Cultivars: Implications for Integrated Pest Management

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ABSTRACT

Capsicum annuum is an economically important crop in Thailand, widely cultivated for culinary, pharmaceutical, and export purposes. However, co-infection by multiple pests, particularly root-knot nematodes (RKN) and spider mites, is increasingly observed under field conditions, forming a complex that severely compromises plant health. In this study, we investigated the interactions of single and mixed infections of root-knot nematodes (*Meloidogyne incognita*, *M. enterolobii*) and spider mites (*Tetranychus kanazawai*) on three commercial Thai chili cultivars: 'Ampawa' (*C. annuum* cv. Ampawa), 'Jinda' (*C. annuum* cv. Jinda), and 'Superhot' (*C. annuum* cv. Superhot). All cultivars were more susceptible to *M. incognita* than *M. enterolobii* ($P<0.05$). Mixed RKN infections resulted in greater disease severity than single infections; the Jinda cultivar showed the highest root gall index (2.5±0.2) and reproductive factor (Rf = 82.5±14.9). Co-infection with both RKN species increased root damage and exacerbated foliar damage caused by *T. Kanazawai*, increasing overall disease severity by 47.49% to 64.16% compared to uninfected controls ($P<0.05$). While Ampawa displayed partial tolerance to spider mite injury under *M. enterolobii* infection (leaf damage percent = 5.0±5.0), this tolerance diminished under mixed RKN infection (83.3±9.6). These findings highlight the synergistic effects of root and foliar pests, underscoring the importance of considering pest interactions in disease management strategies. Understanding cultivar-specific responses to pest complexes is critical for developing integrated management approaches and breeding programs aimed at improving chili resilience subject to multi-pest pressure.

Keywords: *Capsicum annuum*, *Meloidogyne incognita*, *Meloidogyne enterolobii*, *Tetranychus kanazawai,* Pest complex, Disease severity

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INTRODUCTION

Plants in agricultural ecosystems are frequently exposed to simultaneous biotic and abiotic stresses (Zafar et al., 2025). Biotic stressors include pathogens and herbivores, while abiotic factors (such as drought, heat, and wind) further compromise plant health and productivity (Kohli et al., 2013; Zafar et al., 2023). In field conditions, these stressors rarely occur in isolation; plants often face concurrent above- and below-ground attacks (Georgieva & Vassileva, 2023). To cope, plants have evolved sophisticated defense mechanisms, including perception of herbivory and pathogen signals, and interact with organisms that exploit plant tissues (Mahanta & Setia, 2024). Their immune systems can recognize damage self-signals or foreign elicitors, triggering defense cascades (Duran-Flores & Heil, 2016). Notably, nematode-associated molecular patterns (NAMPs) are now recognized as inducers of pattern-triggered immunity against plant-parasitic nematodes (PPNs), such as *Meloidogyne* spp. (Kaloshian & Teixeira, 2019).

Root-knot nematodes (RKNs), particularly *Meloidogyne incognita* and *M. enterolobii,* are among the most destructive PPNs, responsible for over USD 157 billion in

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global agricultural losses annually (Youssef et al., 2013). Their wide host range (>3,000 plant species), high fecundity, and ability to induce gall formation make them formidable pests (Jones et al., 2013). RKNs secrete effectors that modulate plant cellular machinery to form multinucleated giant cells —nutrient sinks that support nematode development (Favery et al., 2016). *M. enterolobii* is regarded as one of the most virulent species due to its aggressiveness, adaptability, and capacity to overcome plant resistance genes (Sikander et al., 2020). However, its morphology is often indistinguishable from *M. incognita*, necessitating molecular confirmation (Boonrin et al., 2024). Infected plants exhibit severe symptoms, including leaf chlorosis, wilting, stunted growth, and extensive root galling (Jia et al., 2022). Crop yield reductions associated with *M. enterolobii* include up to 70% in guava, 50% in cucumber and tomato, and 100% in mulberry (Carneiro et al., 2007). Although *M. enterolobii* is considered highly aggressive, recent studies indicate that *M. incognita* can cause more severe damage in certain crops, including chili (Oyetunde et al., 2022). In naturally infested fields, mixed RKN species are frequently observed, such as *M. javanica-M. incognita* and *M. javanica-M. arenaria* combinations (Devran et al., 2017). Co-infections by multiple *Meloidogyne* spp. have been shown to increase disease severity compared to single-species infections, but such interactions remain poorly characterized in chili (Kayani et al., 2013; Oyetunde et al., 2022). This gap is important in chili production systems, particularly in Southeast Asia where the crop holds high economic and cultural value. Thai chili cultivars are frequently cultivated under open-field conditions with limited pest control infrastructure, making them especially vulnerable to complex pest interactions. Understanding these interactions is thus critical for sustaining yield, informing cultivar selection, and designing cost-effective integrated pest management (IPM) strategies tailored to regional production systems.

Concurrently, plant-feeding mites in the Tetranychidae family also pose major threats to agriculture (Matsuda et al., 2018). *Tetranychus kanzawai* Kishida, commonly known as Kanzawa spider mites, is a notorious pest of papaya, tea, cherry, and strawberry (CABI, 2024). These mites damage leaves by piercing cells and consuming chloroplast content, resulting in stippling, discoloration, and in severe infestations, leaf drop and plant death (Hsu et al., 2015). Their high reproductive rate and resistance to broad-spectrum pesticides necessitate integrated pest management (IPM) approaches, including biological control (Cheng et al., 2009). Chili (*Capsicum annuum* L.) is a valuable horticultural crop in Thailand, cultivated for domestic consumption, medicinal purposes, and export. Cultivars such as Jinda, Ampawa, and Superhot are widely grown for their high yield and market appeal (DAE, 2024). However, chili production is increasingly threatened by pest pressure from both RKNs in the rhizosphere and spider mites on the foliage (Sultana et al., 2023; Boonrin et al., 2024). While these pests often co-occur in the field, their combined impact on plant health and productivity remains poorly understood. Most previous studies rely on controlled environments or single-pest inoculation, which do not simulate real field conditions. There is a need for integrated, multi-pest challenge studies that reflect actual agricultural scenarios in Southeast Asia. Specifically, how co-infection influences pest dynamics and cultivar-specific responses has not been adequately investigated. Additionally, the temporal progression and interaction timing of root and foliar pests (e.g., early root colonization followed by mite outbreaks) are not well understood, though they have important implications for pest monitoring and timing of intervention in IPM. This study evaluates the effects of single and combined infections by *M. enterolobii*, *M. incognita*, and *T. kanzawai* on three Thai chili (*Capsicum annuum*) cultivars. By quantifying disease severity and pest interactions, the findings provide insights to support the development of integrated pest management strategies and breeding programs targeting multi-pest resilience.

MATERIALS & METHODS

Preparation of *Meloidogyne* Species

M. incognita (Mi) and *M. enterolobii* (Me) were obtained from the Department of Plant-Pathology, Faculty of Agriculture, Khon Kaen University, Thailand. Each nematode specimen was inoculated into 3-week-old okra plants to maintain and multiply the nematode inoculum in the greenhouse for 2 months. Infected plants were carefully uprooted and washed under running tap water. The egg masses of each nematode species were carefully teased from infected okra roots into sterile water on a watch glass. After 7-day incubation, second-stage juveniles (J2) hatched from the egg masses were washed with sterilized water and counted to a concentration of 200 $J2mL^{-1}$ for inoculation in the subsequent experiment.

Meloidogyne Species Identification

The identification of *Meloidogyne* species was confirmed following the protocol described by Holterman et al. (2006). In summary, a 0.2mL PCR tube containing 20µL of distilled water and one nematode was filled with 20µL of worm lysis buffer (1mL of the mixture included 176µL of 1M NaCl [A&D Technology, Japan], 176µL of 1M Tris-HCl (pH 8) [A&D Technology, Japan], 508µL of ddH2O [Invitrogen, USA], 100µL of DTT [Merck, Canada], and 40µL of 20mg/mL Proteinase K [Worthington Biochemical, USA]). The mixture was incubated in a PCR thermocycler (MiniAmp plus, Thermofisher Scientific, USA) for 2 hrs at 65°C, followed by an additional 7min at 99°C. Following the extraction of DNA, the ribosomal RNA gene and the cytochrome oxidase subunit II gene were targeted by the primer sets C2F3/1108 (5'-GGTCAATGTTCAGAAA TTTGTGG-3'/5'-TACCTTTGACCAATCACGCT-3') in a polymerase chain reaction procedure (Powers & Harris, 1993). The 15µL PCR reaction consisted of 2µL of DNA template, 4µL of sterilized distilled water, 0.75µL each of forward and reverse primers, and 7.5µL of 2X PCR master mix (Wizbio Solutions, Korea). PCR amplification was carried out under the following conditions: 5min of initial denaturation at 95°C, followed by 35 cycles of

denaturation at 95°C for 1min, annealing at 48°C for 1min, extension at 72°C for 1min, and final extension at 72°C for 7min. The PCR results were subsequently analyzed using fluorescent labeling on a 1.5% agarose gel in 1X TAE buffer (Prime juice; Biohelix, Taiwan). A 100 bp DNA ladder (Biohelix, Taiwan) was used to compare the size of the DNA. The gel was subjected to an electric field of 100 volts for 25min and then visualized using a blue light transilluminator (Blue Pad Dual LED Blue, Biohelix, Taiwan).

Preparation of *C. annuum* Cultivars

C. annuum cv. Jinda, Superhot, and Ampawa were placed on germination test paper and grown for 7 days until the roots had germinated. The seedlings were transplanted into a 108-well nursery tray filled with sterilized peat moss. Two weeks later, each seedling was transferred to a 1-inch-diameter PVC pot filled with 50g of sterilized sand polymer (SAP), which consisted of 20g of sand and 30g of polymer. The plants were maintained in a greenhouse and twice a week received 10mL of Hoagland solution (Hoagland & Arnon, 1938).

Effect of Root-knot Nematodes on the Response of *C. annuum* under Greenhouse Conditions

Each 45-day-old chili seedling cultivar was inoculated with 200 J2s of either Mi, Me, or a mixture of both (Mi + Me) in a 1:1 ratio. This study comprised four treatments for each chili cultivar: Mi, Me, a mixture of Mi + Me and a mock inoculation using sterilized water (control). The experiment was arranged in a random complete block design (RCBD) with 10 replicates for 1 trial. Data on plant growth (height, canopy size, shoot fresh weight, shoot dry weight, root weight, and root length) were recorded every 15 days until the experiment was terminated at 45 days post-inoculation. At the end of the experiment, the chili plants were assessed for root gall index (GI) following the method outlined by Barker (1985): 0 = no galls; 1 = 1–25% galls in the root system; 2 = 26–50% galls in the root system; 3 = 51–75% galls in the root system; 4 = more than 75% galls in the root system. After counting, the roots were chopped into small pieces (~1–2 cm long) and shaken with 0.6% sodium hypochlorite (Clorox, Malaysia) for 4min using a homogenizer. The egg suspension was poured into a series of sieves with 150µm and 25µm apertures and rinsed with running tap water. Nematode eggs retained on the 25µm sieve were collected, counted under an inverted microscope (Olympus CKX53, Japan), and reported as the number of eggs per gram of root weight. The calculated reproductive factor (Rf), following the method of final population (Pf), was divided by the initial population (Pi) (Hajihassani et al., 2019).

Effect of Spider Mite Infection on *C. annuum* Growth under Field Conditions

Each 45-day-old chili cultivar seedling was placed in field conditions (16°28'35.0"N 102°49'13.8" E) where spider mite populations were prevalent. This study contained three treatments (each chili cultivar). The experiment was arranged in a random complete block design (RCBD) with 5 replicates in each of two trials. Data on the percentage of infected leaves due to spider mites was recorded weekly until the experiment was terminated at 4 weeks post-treatment. The formula used for calculating the percentages of damage is:

The percentage of infected plants = (number of infected leaves/total number of leaves) *100

Then, the percentages of damage were compared using the following scoring system to determine disease severity: Level 1 = egg spider mite on chili leaves, Level 2 = leaf curl/white leaves, and Level 3 = plant death (modified from Naituku et al. (2017)).

Effect of Co-infection by *Meloidogyne* Species and Spider Mites on *C. annuum* under Field Conditions

The most susceptible (Jinda) and resistant (Ampawa) chili cultivars were selected for further study on co-infection with RKNs and spider mites. Each 45-day-old chili seedling cultivar was inoculated with 200 J2s, as detailed above. Two days after nematode inoculation, the infected plants were transferred to field conditions. The experiment was arranged using a random complete block design (RCBD) with 4 replicates in each of two trials. In this study, both trials were conducted simultaneously; however, the egg masses of each nematode species were isolated from different infected plants, and the plants were placed in distinct areas of the fields. The data on the percentage of spider mite-infected leaves was recorded weekly (as described above) until the experiment concluded at three weeks post-inoculation. Additionally, the interaction of timing, chili cultivars, and RKN species on the infestation rate of spider mites was determined by statistics in following next section.

Statistical Analysis

Statistical analyses were conducted using the SPSS software version 28.0.1.0 (licensed to Khon Kaen University). One-way and two-way ANOVAs were used to evaluate treatment effects and interactions, respectively. Means were separated using Tukey's Honest Significant Difference (HSD) test at $P<0.05$.

RESULTS

***Meloidogyne* Species Identification**

Meloidogyne species were verified through molecular identification using the primer C2F3/1108. This primer set was specifically designed to distinguish between *Meloidogyne* spp. After DNA amplification, the results revealed two distinct DNA product sizes: 700 bp (Fig. 1) for *M. enterolobii* and 1,500 bp (Fig. 2) for *M. incognita*. Consequently, these results confirmed the species of *Meloidogyne* used in the experiment.

Effect of Root-knot Nematodes on the Response of *C. annuum* under Greenhouse Conditions

Plant growth: Chili cultivars Jinda, Superhot, and Ampawa were inoculated with RKNs, and their effects on plant growth were evaluated at 15, 30, and 45 days after inoculation. No significant difference was observed in all growth parameters across nematode-inoculated treatments, except for the shoot fresh weight of cv. Jinda at 30 days post-inoculation with combined Mi + Me, which

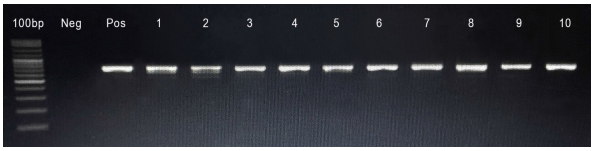

Fig. 1: Agarose gel electrophoresis of the DNA of *Meloidogyne enterolobii* (Me) isolated from the okra amplified by PCR with primers (A) C2F3/ 1108. DNA band size was compared with 100 bp DNA ladder, Lane 1–10: DNA samples from *M. enterolobii* in this study, Lane Neg: negative control (without DNA template), and Lane Pos: DNA sample from *M. enterolobii*.

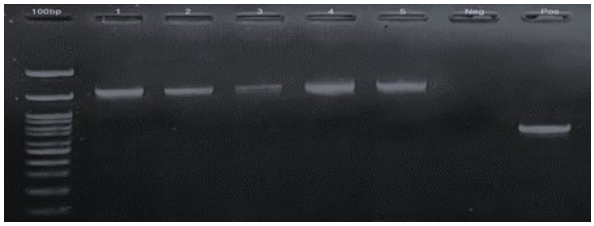

Fig. 2: Agarose gel electrophoresis of the DNA of *Meloidogyne incognita* (Mi) isolated from the okra amplified by PCR with primers (A) C2F3/ 1108. DNA band size was compared with 100 bp DNA ladder, Lane 1–10: DNA samples from *M. incognita* in this study, Lane Neg: negative control (without DNA template), and Lane Pos: DNA sample from *M. enterolobii*

was significantly lower than that of healthy plants (Fig. 3 and 4). In the current study, although several plant growth parameters did not show significant differences, cv. Jinda appeared to have a stronger response than other chili cultivars, particularly in plants infected with a combination of Mi + Me, which tended to exhibit reduced plant height, shoot fresh weight, shoot dry weight, and root fresh weight when compared to those inoculated with either Mi or Me alone. Nematode reproduction: At 45 days following RKN inoculation with either Mi, Me, or a combination of Mi + Me, the number of eggs, nematode reproduction factor (RF), and root galling index were assessed and contrasted with those of the healthy control plants. The results indicated that the Jinda cultivar exhibited greater susceptibility compared to the Ampawa and Superhot cultivars, both of which showed tolerance to RKN. Moreover, for all three chili cultivars, Mi and Mi + Me were more aggressive than Me, as indicated by a higher egg count per gram of root compared to the other treatments. In the Ampawa and Superhot cultivars, plants inoculated with Mi (4,581.72egg/g root) and Mi + Me (2,586.77egg/g root) had the highest number of nematode eggs, whereas Me (83.05egg/g root) exhibited significantly lower egg counts compared to those treatments and was similar to the control (Table 1). Similarly, for the RF value and root galling index, the findings corresponded with those observed for the nematode eggs; specifically, the Ampawa and Superhot cultivars exhibited the highest values when inoculated with Mi and Mi + Me, while Me did not differ from the control. On the contrary, the RF value and root galling index observed in Jinda were significantly higher in all nematode-inoculated treatments compared to the control, particularly in the plants inoculated with Mi.

Overall, these three chili cultivars are considered good hosts for both Mi and Me. However, the Ampawa and Superhot cultivars were more tolerant of Me than of Mi and Mi + Me when compared to the Jinda cultivar. Moreover, the co-infected Jinda cultivar with both *M. incognita* and *M. enterolobii* exhibited a moderate increase in nematode reproduction (egg/g root) compared to plants infected with either *M. incognita* or *M. enterolobii* alone (Table 1). These results reflect those of the plant growth parameters; specifically, shoot fresh weight and shoot dry weight in the plants inoculated with Mi + Me were significantly lower compared to the other treatments (Fig. 2).

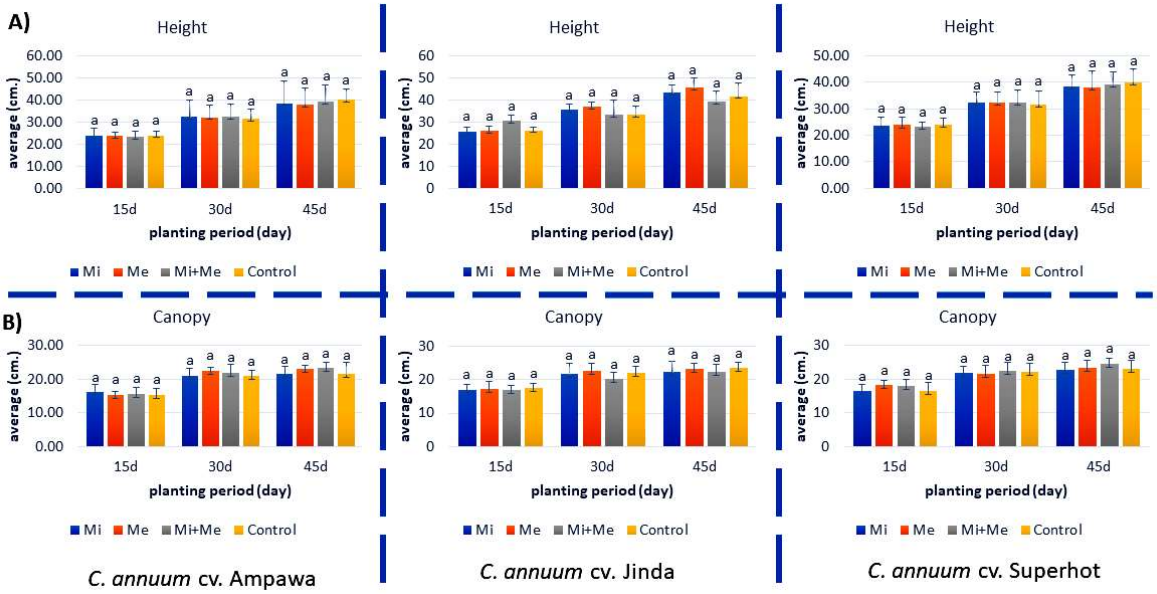

Fig. 3: Effect of root-knot nematode (RKN) infection on the growth parameters of three Capsicum annuum cultivars (Jinda, Ampawa, and Superhot) across different points of time (15, 30, and 45 days post-inoculation). The treatments were Mi = *Meloidogyne incognita*, Me = *M. enterolobii*, Mi + Me = mixed infection of both nematodes, and Control = non-inoculated plants. Plant Height (A) and Canopy Size (B); each graph in (A) and (B) represents a different chili cultivar (Left = Jinda, Middle = Ampawa, and Right = Superhot). Means were compared using Tukey's HSD test ($P<0.05$). Error bars indicate standard deviation (n = 10).

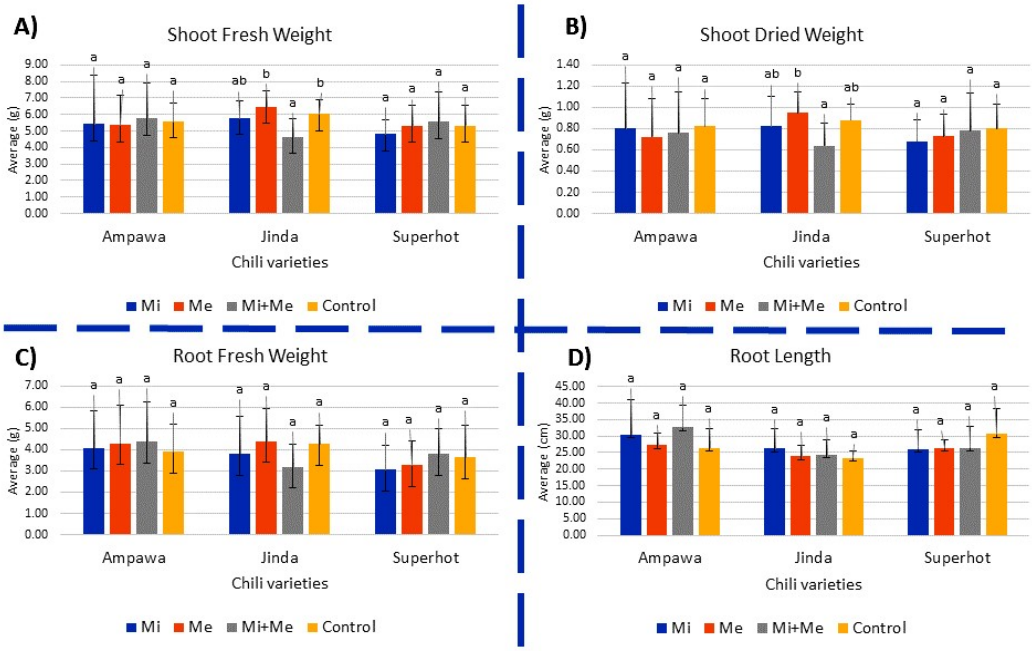

Fig. 4: Effect of *Meloidogyne incognita* (Mi), *M. enterolobii* (Me), and mixed Mi + Me infections on shoot and root growth traits of three *Capsicum annuum* cultivars (Ampawa, Jinda, and Superhot) at 45 days post-inoculation. Parameters measured were: (A) shoot fresh weight, (B) shoot dry weight, (C) root fresh weight, and (D) root length. Different letters above bars indicate statistically significant differences among treatments based on Tukey's HSD test ($P<0.05$). Error bars represent standard deviation (n = 10).

Table 1: Comparisons of the number of root-knot nematode eggs on *Capsicum annuum* cultivars Ampawa, Jinda, and Superhot infected with *M. incognita* (Mi), *M. enterolobii* (Me), and *M. incognita* + *M. enterolobii* (Mi + Me) at 45 days post-inoculation

Treatments	Chili cultivars								
	Ampawa			Jinda			Superhot		
	egg/g root	Rf	GI	egg/g root	Rf	GI	egg/g root	Rf	GI
Mi	4,581.7±1,103.5aB	77.7±13.3aC	1.9±0.2aC	7,590.1±1,279.7aC	129.8±18.5bC	2.1±0.2aC	3,996.9±872.1aB	51.7±9.1aB	2.2±0.2aC
Me	83.1±24.2aA	1.7±0.5aA	0.50±0.2aAB	2,508.9±605.5aAB	48.7±11.3bB	0.9±0.1aB	211.4±114.3aA	2.7±1.2aA	0.5±0.2aA
Mi+Me	2,586.8±684.4aB	47.9±11.5aB	1.00±0.2aB	4,858.2±606.5aB	82.5±14.9aB	2.5±0.2bC	3,258.4±450.3abB	55.0±10.1aB	1.2±0.1aB
control	0.0aA	0.0aA	0.00aA	0.0aA	0.0aA	0.0aA	0.0aA	0.0aA	0.0aA

Mean±SE (n = 10) were compared using Tukey's Honestly Significant Difference (HSD) Test at a 0.05 significance level. Similar lower-case letters indicate that means of each parameter are not significantly different within a row; similar upper-case letters indicate that means are not significantly different within a column. Rf–reproductive factor and GI–Gall index

Effect of Spider Mite Infection on *C. annuum* Growth under Field Conditions

Percentage of feeding damage: The impact of spider mites on *C. annuum* cv. Jinda, Superhot, and Ampawa were assessed under field conditions. The symptoms (egg spider mite infestation, leaf curl/white spot symptoms, and plant death) were monitored weekly over two trials until the experiment was terminated at 4 weeks post-treatment (Fig. 6). At week 1, mature leaves displayed white spots, with spider mites and their eggs found on the chili leaves, affecting an average of 46.37% of the plants. By the second week, mature leaves exhibited complete whitening, along with the presence of spider mites and their eggs. The spider mites spread to the young leaves, which showed symptoms of white spots, with an average of 77.14% of the plants affected. In the third week, the entire plant displayed severe symptoms, including dried leaves that were unable to undergo photosynthesis, stunted growth, and the death of some plants, with an average of 92.07% affected. By week 4 post-treatment, most plants exhibited severe symptoms, and some, particularly the Jinda cultivar, perished, with an average of 95.51% of the plants affected (Table 2). Nevertheless, the percentage of leaves infected by spider mites did not significantly differ among the chili cultivars over the 4-week period. The Jinda cultivar sustained greater damage than the other cultivars, while the Ampawa cultivar showed fewer plant deaths relative to the others (Fig. 4). This study demonstrated that Thai chili cultivars, particularly the commercial varieties, were still susceptible to spider mites, with a greater impact on the Jinda cultivar. Disease severity: The severity of

Table 2: The percentage of infected leaves due to spider mites in different *Capsicum annuum* cultivars (Ampawa, Jinda, and Superhot) at 1, 2, 3, and 4 weeks post-treatment

Weeks after treatment	Percentage of damage by spider mites*			
	Ampawa	Jinda	Superhot	Average
Trial 1				
1	21.67±2.89aA	28.89±7.70bA	53.33±5.77aA	34.63
2	62.5±43.30aA	65.00±36.16abA	38.33±14.53aA	55.28
3	56.25±14.93aA	72.02±25.82abA	75.00±28.87aA	67.76
4	58.33±38.19aA	100.00±0.00aA	61.67±12.58aA	73.33
Trial 2				
1	31.25±4.17aA	27.50±15.00aA	41.25±11.82aA	33.33
2	40.19±34.46aA	41.33±16.09aA	41.67±21.25aA	41.06
3	65.24±32.28aA	40.67±5.96aA	74.00±37.15aA	59.97
4	64.45±3.85aA	44.44±9.62aA	47.78±13.47aA	52.23

Values are mean±SD (n = 4). Means were compared using Tukey's Honestly Significant Difference (HSD) Test at 0.05 significance level. Similar lower case letters indicated that means are not significantly different within a row; similar upper-case letters indicate that means are not significantly different within a column

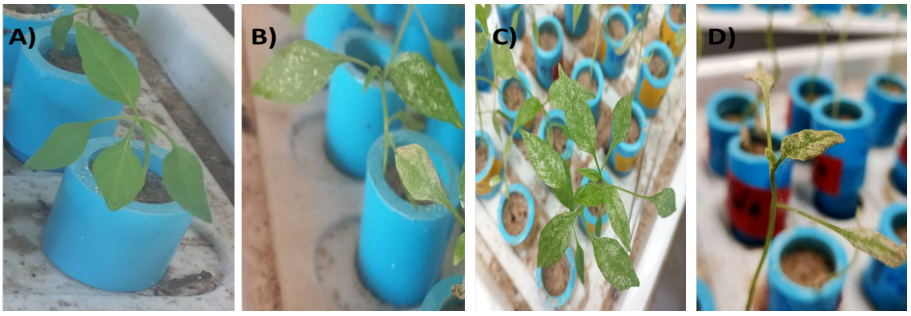

Fig. 5: The severity of disease symptoms caused by spider mites on chili leaves: (A) Healthy chili; (B) and (C) (Level 2) necrosis (white spots) on leaves and yellowing of the plants, with expansion of white spots exceeding 50% of the leaf tissue, leading to discoloration (white leaves); (D) (Level 3) dying leaves and the presence of spider mite webs on the chili plants.

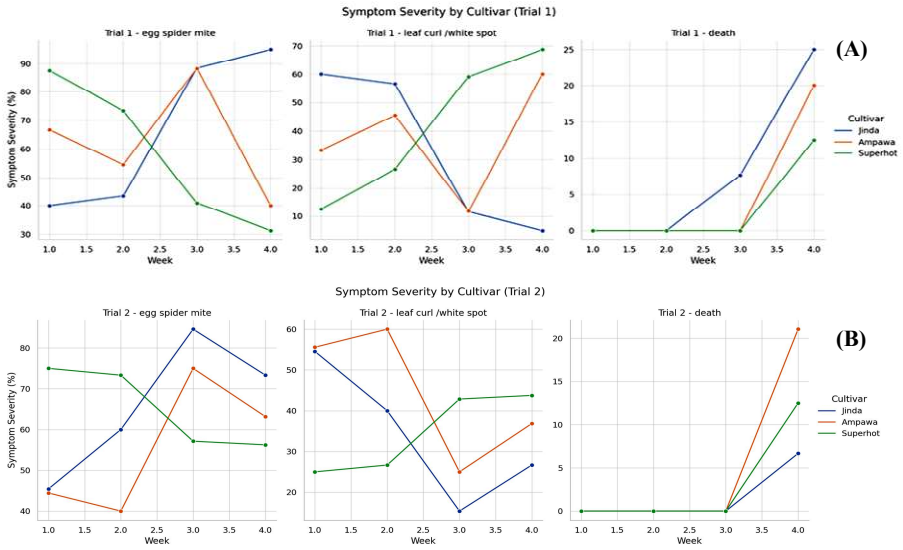

Fig. 6: Symptom severity progression in three *Capsicum annuum* cultivars (Jinda, Ampawa, and Superhot) exposed to natural spider mite (*Tetranychus kanzawai*) infestation under field conditions. Data represent the percentage of severity based on (left) egg deposition, (middle) leaf curl and white spot symptoms, and (right) plant death, assessed weekly over four weeks. Trial 1 (A) and Trial 2 (B) were conducted under similar environmental conditions with different plant batches. Each line represents the mean of five replicates.

symptoms caused by spider mites was divided into three levels: Level 1 was characterized by spider mite eggs on the leaves and yellowing of the plants; Level 2 involved leaf curl and the expansion of white spots in the leaf tissue, leading to discoloration (white leaves); and Level 3 was indicated by dying leaves and the presence of spider mite webs on the chili plants (Fig. 5).

The initial symptoms in the Jinda cultivar included white leaves for approximately 60% of the plants, with spider mites subsequently spreading to new leaves, resulting in white spots and ultimately causing the death of 25% of the plants. In the Ampawa cultivar, the initial symptoms manifested were white spots on the leaves of about 33% of the plants. The spider mites subsequently proliferated to new leaves, resulting in white leaves and the death of 20% of the plants. In the Superhot cultivar, the initial symptoms presented were white spots on the leaves of approximately 12.5% of the plants, resulting in white leaves and the death of 12.5% of the plants, Although the percentage of infected leaves was similar across the chili cultivars, the Jinda cultivar exhibited more pronounced virulent symptoms compared to the others (Fig. 6). Consequently, the study demonstrated that susceptibility response of the various cultivars differed significantly. While all the chili cultivars in the study were susceptible to spider mites, each cultivar exhibited a different response pattern, most likely influenced by factors such as plant cells and genetics. In this study, the spider mites were identified as *Tetranychus kanzawai* Kishida based on their morphological features. The main distinguishing characteristics compared to other species were the absence of wings and antennae, as well as its oval-shaped body. The dorsal border of the aedeagus was typically rounded, featuring a large knob that was approximately twice the length of the neck (Fig. 7).

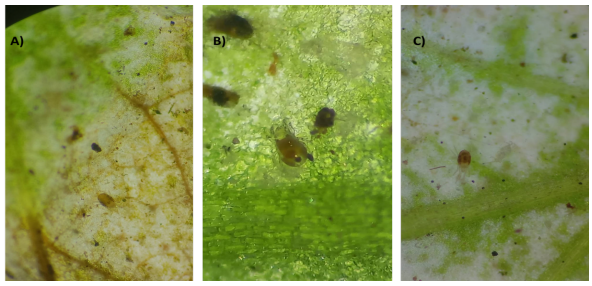

Fig. 7: The morphology of *Tetranychus kanzawai* Kishida on chili leaves, observed under a stereo microscope: (A) Ampawa cultivar, (B) Jinda cultivar, and (C) Superhot cultivar.

Effect of co-infection by *Meloidogyne* Species and Spider Mites on *C. annuum* under Field Conditions

This research was conducted to investigate the synergistic effect of RKN infection and spider mites on disease severity in selected chili cultivars, with a focus on the most susceptible and resistant responses to either RKNs or spider mites. The results in the Ampawa cultivar at week 1 were consistent across two trials—spider mite damage ranged from 4.2% to 83.3%. The most damage was observed in plants inoculated with Mi and those with the mixed infection of Mi + Me. At week 2, the number of

Table 3: Comparisons of the average number of leaves infected by spider mites on two chili cultivars, Jinda and Ampawa, under different root-knot nematode (RKN) treatments, *M. incognita* (Mi), *M. enterolobii* (Me), and their combination (Mi + Me) at 1, 2, and 3 weeks post-inoculation.

week/chili cultivars	Average no. of infected leaves by spider mites							
	Jinda				Amphawa			
	Control	Mi	Me	Mi+Me	Control	Mi	Me	Mi+Me
Trial 1								
1	22.5±9.0bB	58.3±10.8bA	4.2±4.2cB	79.2±12.5aA	37.5±7.5bBC	51.8±15.0aAB	5.0±5.0bC	83.3±9.6aA
2	65.0±20.6abAB	88.1±4.0aAB	39.3±15.8bC	96.4±3.6aA	86.6±5.1aA	83.9±11.8aA	22.0±12.3bB	100aA
3	95.8±4.2aA	100aA	100aA	100aA	100.0aA	95.8±4.2aA	95.8±4.2aA	95.8±4.2aA
Trial 2								
1	13.3±4.5bB	45.8±15.8bB	13.3±8.2bB	85.0±9.6aA	25.3±5.9cAB	55.0±16.6aAB	4.2±4.2bB	74. ±20.4aA
2	45.0±13.5bB	95.8±4.2aA	38.7±13.4bB	100aA	56.5±10.0bB	87.5±8.0aA	20.7±8.7bC	96.4±3.6aA
3	85.7±10.1aA	100aA	100aA	100aA	100aA	96.4±3.6aA	100aA	100aA

Values are presented as mean±SD (n = 4). Means were compared using Tukey's HSD at a 0.05 significance level. Similar lower-case letters indicate that means are not significantly different within a row; similar upper-case letters indicate that means are not significantly different within a column. Average values were calculated using the formula (number of infected leaves/total number of leaves) x 100

Table 4: Interaction analyses of timing (weeks after treatment), chili cultivars, and RKN species on the number of leaves infected by spider mites using two-way analysis of variance

Factor	Trial 1	Trial 2
Timing (A)	*	*
Chili cultivars (B)	ns	ns
RKN species (C)	*	*
A x B	ns	ns
A x C	*	*
B x C	ns	ns
A x B x C	ns	ns

*significant at 0.05 level (ns = not significant)

damages was increased in all treatments. However, in trial 2, the highest percentage of damage was observed in plants inoculated with Mi and Mi + Me, showing a 55% to 71% higher infestation rate of spider mites compared to the control. In contrast, both treatments in trial 1 did not differ from the control. For both trials, no significant difference in spider mite damage across treatments on the Ampawa cultivar was observed 3 weeks post-treatment. Similarly, in the Jinda cultivar, the results were consistent with those observed in the Ampawa cultivar. Specifically, Mi and Mi + Me contributed to the severity of spider mite damage, resulting in higher infestation rates at week 1 of 159% and 252% for trial 1, and 144% and 539% for trial 2, respectively. At week 2 in trial 2, infestation rates were 113% (Mi) and 122% (Mi + Me) higher compared to the control, whereas no significant difference was observed among the treatments and the control in trial 1. Moreover, the current study found no significant difference in spider mite damage in the treatments at week 3 (Table 3).

The inoculation of different RKN species resulted in a different infection rate of spider mites on chili. Both chili cultivars demonstrated that infection with Mi led to higher spider mite infestation rates than Me, while the mixed inoculation of Mi + Me resulted in increased susceptibility to spider mites compared to each single nematode inoculation. Based on the analysis of the two-way ANOVA, timing (weeks after treatment), type of nematodes, and the interaction between timing and type of nematodes were found to have a significant effect on the infestation rate of spider mites (Table 4), while other factors did not have an effect. This finding highlights that chili plants infected by RKN, specifically by mixed RKN species, with a longer incubation period in the field result in a higher percentage of spider mite-infected leaves compared to uninoculated RKN plants.

DISCUSSION

Root-knot nematodes (*M. enterolobii* and *M. incognita*) and spider mites are regarded as the primary pests in chili cultivations (Sultana et al., 2023; Boonrin et al., 2024). Although these pests are widespread in many chili-cultivating areas of Thailand, the resistance levels of chili cultivars to these disease-causing agents have yet to be studied. This study indicates that the chili cultivars Ampawa, Jinda, and Superhot, which are commercially grown in Thailand, are susceptible hosts for *M. enterolobii*, *M. incognita*, and mixed infections of both nematodes, as well as for spider mites. Nematode reproduction factor (Rf = final population / initial population density) is used widely as a crucial indicator for the response of plants to the RKN infestation (Beesa et al., 2022). The interpretation is as follows: Rf > 1 indicates that the host plant is susceptible or a good host, while Rf < 1 suggests that the host plant is resistant or a non-host (Seinhorst, 1967). Additionally, root gall indices (GI) serve as another widely used measure for assessing plant resistance to RKNs (Dong et al., 2007). Based on the findings, the Rf values for *M. incognita* and *M. enterolobii* observed in the three studied chili cultivars are greater than 1. Surprisingly, *M. incognita* more aggressively damages all chili cultivars than *M. enterolobii*. Among these, the Jinda cultivar exhibited the highest susceptibility, showing the highest Rf values for both nematodes. This result contrasts with several reports indicating that *M. enterolobii* is more virulent to plant hosts than *M. incognita* (Bui & Desaeger, 2021; Dareus et al., 2021). This discrepancy might be attributed to the specific genetic backgrounds of the Thai chili cultivars used, or possibly the unique environmental conditions of this experimental setup, as host susceptibility is known to be influenced by genotype, initial RKN population densities, and environmental factors (Starr & Mercer, 2009b; Kayani et al., 2017). Moreover, the mixed infection of both species increased RKN invasion in chili more than inoculation with either *M. incognita* or *M. enterolobii* alone. The impact of co-infection by both nematodes on chili disease severity has not yet been studied or documented.

However, similar studies have shown that co-infection with *Meloidogyne* species leads to greater damage than infections caused by individual RKN species (Kayani et al., 2017; Oyetunde et al., 2022). A host's susceptibility is determined by genotype, initial RKN population densities,

and environmental factors (Starr & Mercer, 2009a; Kayani et al., 2017). The resistance of chili cultivars to root-knot nematodes results in reduced reproduction and suppression of nematode penetration, leading to a localized hypersensitive response in the host plants (Pegard et al., 2005; Williamson & Kumar, 2006). Moreover, certain chili cultivars carry dominant resistance genes that are effective against several species of root-knot nematodes. For example, the N gene in resistant genotypes of *C. annuum* and *C. chinense* has been effective against *M. incognita* (Thies, 2011). Additionally, peppers with both the *Me1* and *Me3* genes, originally discovered in two separate breeding lines, provide consistent resistance to the three primary species *M. arenaria*, *M. incognita*, and *M. javanica* (Hendy et al., 1985; Hajihassani et al., 2019). Furthermore, some *Capsicum annuum* L. cultivars are resistant to *Mi* due to the presence of the N gene, which has also been shown to resist *M. javanica* and *M. arenaria* (Maquilan et al., 2020). There is evidence that this gene's function is activated and upregulated during the early stages of nematode infection, leading to the induction of resistance pathways (Kumar et al., 2019). Downregulated genes exhibit significant differential expression and are associated with susceptible pathways (El-Sappah et al., 2019; Hu et al., 2020).

However, the specific resistance genes present in the Thai chili cultivars used in this study remain unknown. The observed susceptibility of Ampawa, Jinda, and Superhot suggests either the absence of these known dominant resistance genes in these commercially grown Thai cultivars or that the specific *M. incognita* and *M. enterolobii* populations in this study may have overcome these resistance mechanisms. Further genetic characterization of these chili cultivars would be beneficial to elucidate the underlying reasons for their susceptibility. In assessing the severity of chili responses to spider mites, damage levels of the chili leaves were observed at three levels across three chili cultivars. This study revealed that the spider mite (*Tetranychus kanzawai*) damaged three chili cultivars, inflicting approximately 50% feeding damage in the first week. This damage escalated to greater than 90% by the third week, culminating in significant deterioration and eventual plant decline under the examined environmental conditions.

Several studies have evaluated the resistance of maize, beans, and chili to spider mites (Chacón-Hernández et al., 2020; Franzin et al., 2020); however, there is no information regarding the response of chili cultivars in Thailand. This is the first report of the susceptible reactions of the chili cultivars Ampawa, Jinda, and Superhot to spider mites in Thailand. The Jinda cultivar appeared more susceptible than the other cultivars tested, a finding that aligns with general observations of the *C. annuum* plant's susceptibility to spider mites. While severe damage is common in cultivated *C. annuum* in Thailand, wild chili types often serve as genetic resources due to their natural resistance, which may stem from the production of secondary metabolites such as phenolic compounds, capsaicinoids, and volatile compounds, or specialized physical structures such as trichomes and waxes that deter feeding and oviposition (Smith, 2005; Hayano-Kanashiro et al., 2016; Chacón-Hernández et al., 2020). The presented resistance is of the antibiosis type, which resulted in increased nymph mortality and decreased fecundity (Oliveira et al., 2018).

This study showed detailed changes in feeding damage symptoms of spider mites for each cultivar. Specifically, the research showed that plants react differently to spider mite attacks over time—from the early feeding stages until the plant's death. This is because each plant variety has different pattern recognition receptors (PRR) that detect the signals from spider mites, known as herbivore-associated molecular patterns (HAMPs), and trigger plant defenses. This process involves a series of signal transmissions influenced by hormones such as jasmonic acid (JA), salicylic acid (SA), and ethylene (ET). As a result, genes are reprogrammed, and transcription factors are activated to produce defensive molecules against pests. Additionally, the plants release compounds that attract natural enemies of the spider mite or communicate with nearby plants (Santamaria et al., 2013; Agut et al., 2018; Stahl et al., 2018; Erb & Reymond, 2019; Santamaria et al., 2020).

Spider mites can suppress plant defenses through three main strategies—avoidance, metabolic resistance, and suppression (Kant et al., 2015; Blaazer et al., 2018). They achieve this through digestion, detoxification, and transport of xenobiotics using enzymes such as cytochrome P450, carboxyl/cholinesterases (CCEs), glutathione S-transferases (GSTs), and ATP-binding cassette transporters (ABC) (Grbic et al., 2011; Wybouw et al., 2018). The most interesting strategy is the suppression of defenses, where spider mites act downstream of the phytohormone pathway to suppress SA- and JA-dependent defenses in tomato plants. It is worth noting that each mite strain affects the expression of tomato defense genes differently (Alba et al., 2015). In the experiment involving co-infection between RKN and spider mites, this study demonstrates that the severity of damage caused by spider mites significantly increased in plants inoculated with two species of RKN, particularly in the Jinda and Ampawa cultivars. The findings indicate that a mixed infection of both RKN species triggers more severe spider mite attacks compared to the inoculation of a single RKN species on either chili cultivar, with damage levels around 2 to 4 times higher in the Jinda and Ampawa cultivars than in RKN-uninoculated plants. In mixed infections, it is plausible that the combined stress from two *Meloidogyne* species induces greater systemic immunosuppression or alters host physiology in spider mite proliferation, potentially by synergistically affecting plant nutritional quality or defense signaling pathways.

While previous experiments did not confirm the impact of mixed infection between Mi and Me (Dareus et al., 2021; Mbaluto et al., 2021; Ripa et al., 2023), this experiment demonstrated that such mixed infections of RKN affect the percentage of feeding damage caused by spider mites, even when the RKN species are present individually. Root infection by RKN influences leaf anti-herbivore defense and the plant's resistance to spider

mites (Ripa et al., 2023). This assumption is similar to the plant-mediated interaction between Mi and *Spodoptera exigua*, which revealed that Mi root infection systemically affected the plant responses related to jasmonic acid, salicylic acid, and abscisic acid. This suggests that specific leaf responses triggered systemically by the nematode at different life-cycle stages underlie the differential impact of Mi (Mbaluto et al., 2021). Additionally, RKN infection can alter plant molecules, affecting herbivores like tobacco roots and increasing the weight of *Trichoplusia ni* and *S. exigua* larvae (Kaplan et al., 2008).

The infection cycle of RKN plays a crucial role in influencing the interactions between RKN and herbivores when they share a host plant. This is not surprising because how plants interact with RKNs changes significantly from early to later stages of the infection cycle, making it a dynamic process. This suggests that the assistance provided by Mi during the gall stage may have been due to an increase in leaf nutritional quality or suppression of the plant's ability to effectively defend against *S. exigua* (Mbaluto et al., 2021). Notably, the findings emphasize the urgent need for integrated pest surveillance and early detection protocols, as delayed management may allow pest populations to amplify one another's effects. Breeding programs should prioritize resistance screening under simulated multi-pest conditions to ensure resilience under real-world scenarios. This study represents the first report of the synergistic effect of two species of root-knot nematodes (*M. incognita* and *M. enterolobii*) and spider mites (*T. kanzawai*) on chili plants (*C. annuum* L.) in Thailand. The findings revealed that RKN infection, specifically by mixed infection of *M. incognita* plus *M. enterolobii*, increased the feeding behavior of *T. kanzawai*. Surprisingly, the evidence showed that *M. incognita* alone was more harmful to chili plants than *M. enterolobii* across all studied chili cultivars. This was attributed to a higher number of eggs per gram of root, a higher reproduction rate, and a greater gall index. However, further studies are needed, particularly investigations into the expression levels of resistance genes in chili plants infected by RKN, spider mites, or a combination of both, to gain a more comprehensive understanding of their interaction.

Conclusion

This study highlights the high susceptibility of three Thai chili cultivars to *M. incognita*, *M. enterolobii*, and spider mites (*T. kanzawai*). Of most significance, co-infection with *M. incognita* and *M. enterolobii* led to increased spider mite infestation, particularly in the Jinda cultivar, which sustained more severe damage than the Ampawa cultivar. The interaction among chili plants, root-knot nematodes, and insect herbivores represents a crucial yet underexplored area of research. These findings offer valuable implications for integrated pest management and chili breeding programs aimed at enhancing multi-pest-resistant cultivars. Future studies should focus on long-term field monitoring under multi-pest stress to ensure sustainable pest management strategies. An in-depth investigation of resistance-related gene expression and enzyme activity changes is also recommended to enhance understanding of the molecular mechanisms underlying plant defense.

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