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Original Research

Spread of the Tomato Russet Mite, *Aculops Lycopersici* (Tryon) (Acari: Eriophyidae) in Geothermal Greenhouses of Cherry Tomatoes and Its Control with the Entomopathogenic Fungi *Beauveria Bassiana*

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Abstract

Geothermal tomato is a very important agricultural development axis in southern Tunisia. The tomato russet mite, *Aculops lycopersici* (Tryon), is a major pest that attacks tomato crops in heated greenhouses, causing economic losses. The present study focused on monitoring the spread of *A. lycopersici* between March and June in a heated tomato greenhouse and testing the pathogenicity of two strains of the entomopathogenic fungus *Beauvaria bassiana* R444 and ATCC in comparison with sulfur, oxygen peroxide, and abamectin. The wind has been shown to spread these mites inside the greenhouse. The mites then spread along the stem of the plant, depending on the number of infected leaves and tomato bunches. The rate of propagation on the stem is linked in the upward direction following the application of the leaf removal technique, which is positively correlated. Treatments with *B. bassiana* against mobile forms of *A. lycopersici* showed reduction rates between 77.57% and 95.45% after 10 days of treatment. Similarly, the other treatments showed efficacy, with significant percentages of reduction of mobile forms equal to 82.19%, 87.58%, and 44.26% for abamectin, oxygen peroxide, and sulfur, respectively. In conclusion, entomopathogenic fungi have proven to be highly effective in reducing *A. lycopersici* populations. These microbiological agents can be considered valuable resources in the fight against this pest, helping to minimize its impact on the ecosystem.

Keywords: Tomato russet mite, dissemination, alternative methods, biological control, chemical control

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Introduction

The tomato russet mite (TRM), *Aculops lycopersici* (Tryon) (Acari: Eriophyoidea), feeds on various plant species of the solanaceae family, including several wild species such as black nightshade (*Solanum nigrum* L.) and cultivated plants such as pepper (*Capsicum annuum* L.), eggplant (*Solanum melongena* L.), potato (*Solanum tuberosum* L.), and tomato (*Solanum lycopersicum* L.) [1]. It is one of the most economically important pests of tomato crops, both in open fields and in greenhouses worldwide [2, 3].

Early detection and monitoring of this pest in tomato crops is complex due to its small size of 150-200 µm [4]. All mobile forms of TRM feed on epidermal cells with short needle-like stylets, about 10–15 µm long, stems, and petioles [5]. These mites inject saliva into the plant cell and suck its contents [6]. It destroys the epidermal cells in tomatoes and forms a thick layer of tissue with a high content of lignin [7]. This pest can complete a generation in one week. It causes extensive leaf browning and defoliation, followed by a significant decrease in yield [8]. Early symptoms can be misdiagnosed as nutritional deficiency, plant disease, or water stress [9]. It causes irregular or wavy wrinkling of the edges of the leaflets, followed by a bronze discoloration and a burnt appearance, which eventually leads to plant drying and death. It causes more damage to immature fruits than to ripe fruits when the density is too high on the plants [10]. Detection and monitoring of *A. lycopersis* is often a problem, as critical densities of mites may not be detected or underestimated [11, 12]. *A. lycopersici* showed reduced or interrupted activity at temperature extremes of 8ºC and 39ºC, with successful development between 11ºC and 36ºC on tomato leaves, highlighting key parameters of its life cycle and reproduction as a function of temperature [13].

Chemical control of TRM is difficult due to many factors, such as this mite hiding under trichomes, making it difficult to reach, especially when the spraying technique is not appropriate [10]. Alternate use of botanical pesticides with other insecticides shows efficacy against this mite without any adverse effects on natural enemies under field conditions [14]. Entomopathogenic fungi (EPF) are s more sustainable alternative to chemical pesticides, which do not affect natural enemies [15]. For example, predatory mites *Phytoseiulus persimilis* and *Neoseiulus californicus* were much less susceptible than adults *Tetranychus urticae* to the EPF *Metarhizium brunneum* V275 [16]. EPFs are effective biological control agents for a wide range of phytophagous mites [16], but none have been identified for controlling populations of TRM.

It is becoming necessary to know the presence of this pest inside geothermal greenhouses in order to determine the right time to intervene to combat it with entomopathogenic fungi, given their effectiveness, and to have other environmentally friendly solutions. The aim of this work is to find out the mode of propagation of the TRM population in tomato crops under heated greenhouses in southern Tunisia and to test the efficacy of the EPF *Beauvaria bassiana* R444 strain (Bb-Protec®) and *Beauvaria bassiana* ATCC strain (Naturalis®) against this mite compared to oxygen hydroxide (Huwa-San TR-50), sulfur (Agrosoufre), and abamectin (Vertimec®).

Material and Methods

Experimental Greenhouse

The spread of TRM was monitored in a greenhouse covering an area of four hectares within a tomato exporting company in the Chenchou sector in the El Hamma region of Gabes governorate (latitude 33º52'21'' North; longitude 9º52'34'' East, altitude 78 meters) between March and June 2022. This multi-tunnel greenhouse was covered with plastic. It also has screened ventilation over the full length of the greenhouse, with an opening capacity of 1.8 m on each side. The tomato crop is conducted soilless using coconut fiber. The cultivated cherry tomato is the hybrid cultivar Sweetelle grafted on the rootstock "DRO141 TX". Planting density is four plants/m^2.

Since cultivated tomatoes have indeterminate growth, they mainly require maintenance, including trellising, stripping, pruning, and harvesting. Trellising consists of keeping the stem vertical by manually rolling the plant around the string passing through the internodes. When the plants reach the trellis hooks attached to the culture string, they are lowered and placed obliquely in one line in the opposite direction from the other to maintain the heads of the plants at the same level of height. Pruning consists of the manual removal of axillary buds that start in the axils of each leaf at the top of the plant. Harvesting consists of picking the fruits that have reached maturity.

Dissemination of Aculops Lycopersici

Symptoms of TRM, detected on various parts of the tomato plant (stems, leaves, and fruits) through visual diagnosis, occurred naturally, leading to the initiation of monitoring its spread in the greenhouse. The first symptoms are represented by light chlorosis on leaves or light gray and brown shades on the stem [11]. The locations of infested plants in the greenhouse were noted each week. In this greenhouse, 30 tomato plants were selected based on a visual variation in TRM densities (i.e., some plants were heavily infested, while others were visually not infested). The following factors were monitored: percentage of infected leaves, percentage of the infected bunch, spread height of TRM on the stem (cm), and development of mobile forms of this mite.

On the trellis wire, the last point the plant reached is marked with a felt-tip pen. After one week, the distance between the marked point and the top of the plant was measured with a tape measure, and the height reached by the plant was again determined to measure the growth of the stem. In addition, the spread height of TRM on the stem (cm) was measured in the same way, but the labeling was done at the highest point of infection.

Leaves were considered infested when they were silvery, bronzed, or dry. Infested leaves (%) = (Number of infested leaves/total number of leaves) ×100. Bunches of fruit were considered infested when the fruits were often smaller and had extensive areas that looked a little like cork or were even cracked. Bunches of fruit infested (%) = (Number of bunches infested/Total number of bunches) ×100.

The development of mobile TRM forms was monitored by sampling five leaflets from each of the 30 affected tomato plants. Mobile forms of TRM was inspected using a binocular microscope (Leica® EZ4E model) on 1 cm^2 of each leaflet.

Pathogenicity of EPF against Aculops Lycopersici

The trial was carried out in another tomato greenhouse (with the same structure, variety, and growing conditions) on three blocks divided into 6 elementary plots (60 plants per plot). Each elementary plot received one of the five treatments, while the sixth elementary plot in each block served as an untreated control. The monitoring of climatic conditions inside the greenhouse was carried out during the experimental period. The temperature was relatively high, ranging from 17ºC to 44ºC, with relative humidity varying between 60% and 95%. The application of the products was carried out on May 12, 2022, with a back spray. The main characteristics of the commercial products tested against TRM are presented in Table 1.

Sixty tomato leaflets were randomly sampled for each treatment on the same day immediately before treatment, and then 3 more samples were taken 3, 7, and 10 days after treatment on untreated and treated plants. Sampled leaflets are examined using a binocular microscope (Leica® model EZ4E), and all mobile forms are counted [17]. The efficacy of each treatment against mobile forms of TRM on tomato leaves was evaluated by applying a reduction rate according to the formula [18]: TR (%) = (P1-P2) / P1 * 100. When: P1 = density of TRM in the control block; P2 = density of TRM in the treated block; and TR = rate of reduction in the level of TRM mobile forms after treatment.

Statistical Analysis

The data has been normalized. The analysis of variance (ANOVA) of the reduction rate of mobile forms of TRM after the treatments were subjected to a unidirectional analysis associated with a Duncan test ($p<0.05$) using XLSTAT 2019 and Microsoft Excel 2013.

Results and Discussion

Dissemination of TRM in Greenhouses

The arrangement of TRM-infected plants in a tomato greenhouse showed that the first symptom appeared in rows of tomatoes located below the openings. Over successive weeks, the number of infected plants increased along the same row. Subsequently, the infestation began to appear in the rows of adjacent tomatoes and spread along these rows in the same way.

At the start of monitoring this pest, the percentage of infected leaves was low, as it did not exceed 13% during March and April. In addition, the infection rate of fruit bunches was as low as 4% per plant (Fig. 1b). The spread height of TRM on the stem was less than 5.30 cm (Fig. 1c). With the beginning of May, the rate of infestation of leaves and fruits began to increase, which in turn coincided with an increase in the infestation of tomato stems. Thereafter, the propagation height of TRM on the stem became almost equal to the growth speed of the stem of the tomato plant. This increase in the severity of the infection coincided with the increase in the maximum temperature inside the greenhouses, which reached 40.1 degrees on May 4, 2022 (Fig. 1a).

On May 13, the spread of TRM at the stem level started to increase until it reached 43.53 cm, in coincidence with the decrease in the rate of infested leaves. The level of spread on the stem was related to the number of infested leaves per plant. The leaf removal works that minimize the number of infested leaves explain this. Similarly, when the number of

Table 1. Characteristics of Commercial products used for the experiment.

Commercial name	Active ingredient	Concentration of active ingredient, type of formulation	Doses
Bb-Protec®	*B. bassiana* strain R444	$\geq 2\times 10^9$ spores /gram, WP	1 g/l
Naturalis®	*B. bassiana* strain ATCC 74040	2,3 x 10^7 spores/ml, SC	1 ml/l
Huwa-San TR-50	Hydrogen Peroxide	50%, LC	2000 ppm
Vertimec®	Abamectin	18 g/l, EC	75 cc/hl
Agrosoufre	sulfur	80%, WG	600 g/hl

leaves infested by TRM decreases, the number of infested clusters per plant increases. The evolution of mobile forms of TRM per 1 cm^2 of tomato leaflet and the percentage of infested leaves over time are shown in Fig. 1d). At the beginning of the monitoring, the average number of mobile forms increased progressively over time to reach a peak at the third week of monitoring with 33.7 individuals per cm^2 (Fig. 1d). Following the leaf removal operations, most of the infested leaves were eliminated, which explains the reduction of the mobile forms on the sampled leaflets. Then, the number of mobile forms started to increase in June, reaching 31.19 individuals per cm^2.

In this study, which focused on monitoring the spread of TRM under conditions of natural infestation in a heated tomato greenhouse in southern Tunisia, we observed that the initial symptoms of this mite were detected in the rows of plants situated near the ventilation openings, likely due to the influence of the wind. The spread of this mite from one plant to another can be attributed to several factors. In practice, the trellising of tomato plants facilitates propagation by aligning the direction of the plants in a row. Pruning, leaf removal, and harvesting are also other reasons for infesting other plants in the greenhouse. This is confirmed by other studies, that show that passive spread occurs via

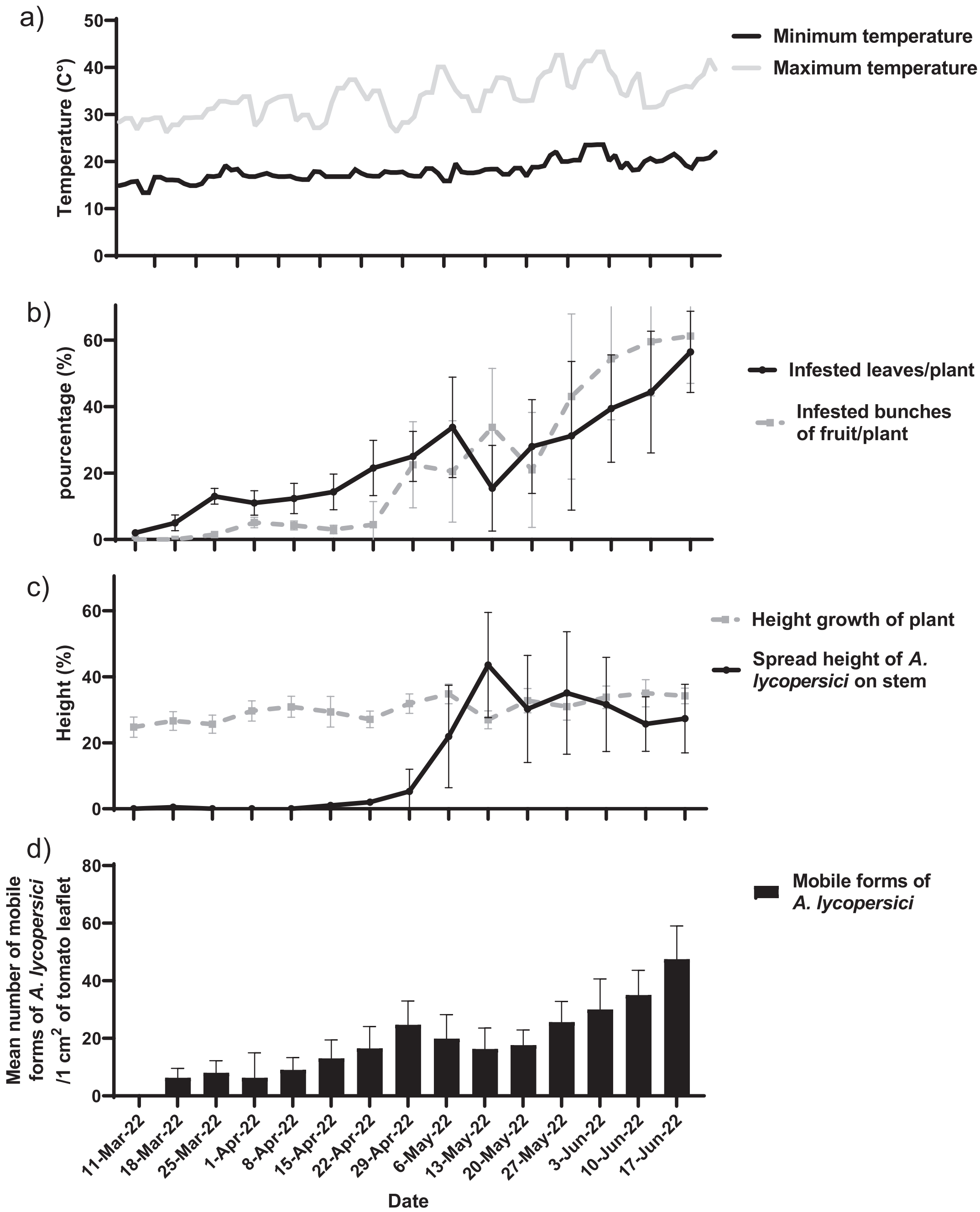

Fig. 1. Spread of TRM over time; a: Temperature in the greenhouse, b: Percentage of infested leaves and bunches, c: Spread height of TRM on stem as a function of the growth speed of the tomato plant, d: Evolution of mobile forms of TRM per 1 cm^2 of tomato leaflet.

air currents and crop management practices [10, 19]. A previous study showed that TRM were initially found on the stem of the plants and progressively attacked parts of the tomato, which explains the increase in their rate of spread on the stem [20]. In addition, TRM generally has an irregular distribution in the tomato field, and the level of aggregation of the group depends mainly on its habits and environmental factors [21].

TRM usually swarms on the upper side of the leaves. On petioles, the density of the mite was high on the lower side. Mite numbers are high on leaflets near the stem. It can be deduced that the infestation starts by rapidly colonizing the leaves, which turn pale green and yellow, then dry up and fall off. On the contrary, the spread on the stem seems to be very slow, not exceeding 5 cm at the beginning of the monitoring period. Leaf stripping applied to favor the generative plant allowed the TRM to increase the speed of colonization of the stem. Our result is similar to the studies which show that TRM colonies swarm on the stems of host plants but also occupy leaf blades, petioles, and fruits [19]. In the study we carried out, we discovered that this mite moves from bottom to top, i.e., towards leaves and tender stems to feed. On the other hand, we noticed that the presence of leaves on the stem reduces the speed of its upward spread, i.e., it moves first to leaves and fruit clusters. Similarly, a study has shown that individuals present a very strong negative geotropism, and the infestation always progresses from the bottom to the top [19]. Thus, when a stem has a downward bend, the TRM concentrates at this angle and no longer moves toward the apex of the plant.

EPF Control Trial against Aculops Lycopersici

The effect of two EPF strains, *B. bassiana* R444, and ATCC, on mobile forms of TRM in the greenhouse of cherry tomatoes by comparing sulfur, abamectin, and oxygen peroxide is shown in Fig. 2. *Beauvaria bassiana* strain R444 and *B. bassiana* strain ATCC showed significant efficacy against mobile forms of *A. lycopersici,* as the reduction rate was 73.86% and 70.03% after 3 days of treatment, respectively. When compared with sulfur, which is most commonly used in tomato greenhouses against TRM, both strains were significantly more effective ($P<0.0001$). Similarly, oxygen peroxide and abamectin caused a significant reduction against mobile forms of TRM of 87.58% and 82.19% after 10 days of treatment. The reduction rates of mobile forms were equal to 77.57% and 95.45% for *B. bassiana* R444 and ATCC at the last sampling date.

Although the use of EPF has been shown to be very effective in reducing the populations of TRM, which can be a good alternative to chemical treatments, forms of sulfur have been commonly used to control the TRM [22]. Nevertheless, in our study, the low percentage of reduction in mobile forms of TRM following the application of sulfur varies between 34.62% and 44.26%, which can be explained by the specific environmental conditions in the greenhouse during the trial period. High temperatures may decrease the effectiveness of sulfur in reducing this mite. Other research underlines the importance of early sulfur application to achieve effectiveness, but the success of this treatment depends heavily on the timing of application and likely unfavorable spring temperatures [23]. Sulfur is an effective acaricide against Eriophyidae; however, its applications have been found inadequate in controlling TRM infestations, and there is a possibility of evolving tolerance in certain regions [10]. Mycoacaricides have been a component of integrated mite management to replace the synthetic acaricides currently used [24]. The treatment with EPF, *Beauveria bassiana*, and *Lecanicillium muscarium* can be used to control *Tetranychus urticae* in protected and geothermal crops, as it is highly effective on eggs and mobile forms [17]. Similarly, another study demonstrated the pathogenicity of the mycopesticide "Naturalis L," based on *B. bassiana* (ATCC 74040), against *Phyllocoptruta oleivora* (Acarina: Eriophyidae) under controlled conditions. In addition, certain strains of *B. bassiana* (strains BB 1.1 and BB 11.6) and *Metarhizium anisopliae* (strain MA 10.1) were pathogenic against another mite of the Eriophyidae family, *Phyllocoptes gracilis* [25].

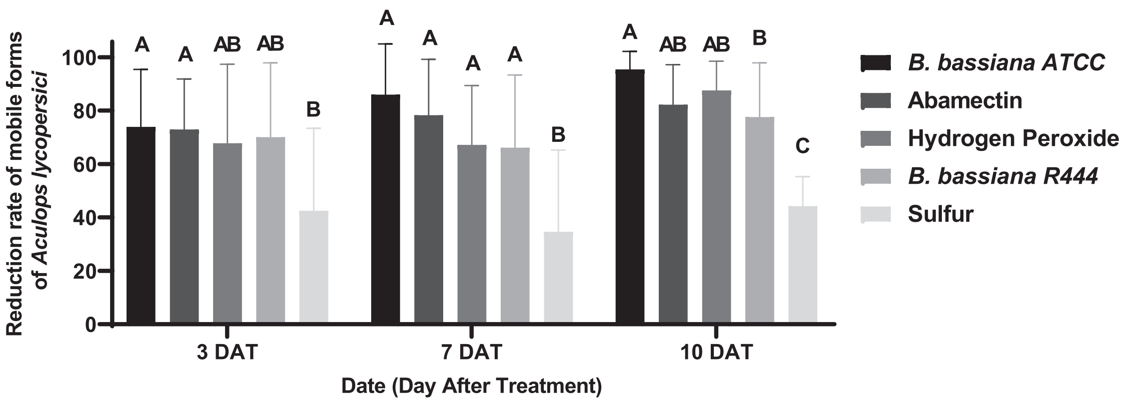

Fig. 2. Reduction Rate of TRM population (mobile forms) after treatment. Means followed by the same letter are not significantly different by Duncan's test ($p<0.05$).

Furthermore, the survival of TRM may have been influenced by a number of entomopathogenic fungi, such as *M. anisopliae* (Metschinkoff), Sorokin, and *Hirsutella thompsonii* (Fisher) [26]. In addition, oxygen peroxide and abamectin were even more effective than EPF in reducing TRM on tomato plants [20], indicating that over 99% of TRM mortality was achieved by applying abamectin. Eriophyoid mites showed sensitivity to abamectin compared to Tetranychidae [27]. Another study showed Huwa-San TR50 to be very effective in reducing all stages of TRM without side effects on the associated predatory mite, *Neosiulus cucumeris*, under greenhouse conditions [28].

Furthermore, climatic conditions are very important for the proliferation of entomopathogenic fungi, particularly the effects of temperature and humidity. This may explain why entomopathogenic fungi are more effective than sulfur. Several studies have shown the importance of temperature in EPF germination, growth, and virulence [29, 30]. In addition, *B. bassiana* ATCC 74040 was more effective than *B. bassiana* R444, which can be explained by the type of formulation. For example, Naturalis® is an oil-based suspension concentrate, while R444 is a wettable powder. Formulation techniques have been shown to increase the viability of entomopathogenic fungi under stressful environmental conditions in the field. For example, oil-based emulsifiable formulations increase fungal tolerance to elevated temperatures [31].

Conclusions

Tomato plant management techniques are the main factors contributing to the spread of *A. lycopersici* in heated glasshouses. The leaf removal technique leads to an increase in the spread of this mite on the stem, which becomes equal to the growth height of the stem in June. Treatments with EPF *B. bassiana* resulted in a significant reduction in the mobile forms of TRM. Consequently, the use of *B. bassiana* as an acaricidal agent could form the basis of integrated control of this mite. This potential opens the way to a more sustainable approach to *A. lycopersici* management, in line with the principles of environmentally friendly, biodiversity-friendly agriculture.

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Conflict of Interest

The authors declare no conflict of interest.

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