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The two-spotted spider mite Tetranychus urticae prefers soybean plants with higher nutritional quality

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Abstract

Microorganisms and arthropods may interact indirectly through plant-mediated mechanisms. Among these microorganisms, phosphate-solubilizing bacteria, commonly used as bioinoculants, have been widely employed to improve phosphorus fertilization efficiency. However, the effects of these inoculations on plant defense mechanisms and herbivory remain inconsistent, varying according to plant species and nutritional status. We aim was to evaluate whether the application of different bioinoculants to soybean plants influences host preference, development, oviposition, and population growth of the two-spotted spider mite, *Tetranychus urticae*. Soybean plants were subjected to the following treatments: (i) BRM 119 (*Bacillus megaterium*) + BRM 2084 (*Bacillus subtilis*); (ii) BRM 119 + BRM 2084 + *Bradyrhizobium* sp.; (iii) *Azospirillum* sp.; (iv) *Azospirillum* sp. + BRM 119 + BRM 2084; (v) fertilization with 0.375 g NPK (control); and (vi) fertilization with 0.750 g NPK.. Adult female *T. urticae* exhibited a greater preference for bioinoculant-treated plants than for control plants. In addition, inoculated plants supported higher population growth of the mite. Inoculation with *B. subtilis* reduced developmental time and increased oviposition. However, the total number of individuals was not affected by the treatments. These findings suggest that the application of bioinoculants may increase the attractiveness of soybean plants and enhance the biological performance of *T. urticae*, possibly as a result of improved host plant nutritional quality. Therefore, the adoption of these bioinoculants should be accompanied by appropriate monitoring and management strategies for the two-spotted spider mite in soybean crops.

Introduction

Microbial communities associated with the plant rhizosphere exhibit high functional diversity and are composed of beneficial microorganisms, including mycorrhizal fungi, nitrogen-fixing bacteria (rhizobia), and plant growth-promoting bacteria (PGPB) (Hasan et al., 2024). These organisms establish through symbiotic and associative interactions, actively playing in mineral nutrition, plant growth, and plant resilience of cope biotic and abiotic stresses. (Alzate Zuluaga et al., 2024; Hnini et al., 2025).

Beneficial microorganisms can modulate plant defense mechanisms against pathogens and herbivores, influencing both direct and indirect plant responses (Disi et al., 2018). Among the main physiological effects attributed to PGPB are enhanced nutrient availability and uptake, as well as the biosynthesis of phytohormones, including auxins (such as indole-3-acetic acid -IAA), cytokinins, and gibberellins (Pineda et al., 2020).

Interactions between microorganisms and arthropods occur predominantly through indirect pathways mediated by changes in host plant physiology and metabolism, which may affect herbivore behavior, development, and performance (Khaitov et al., 2015). Among the microbial groups commonly associated with plants, species of the genus *Bacillus* have received considerable attention due to their ability to produce siderophores and enhance phosphate solubilization, thereby promoting plant growth and development (Chandwani et al., 2023). Species of this genus have frequently been applied in combination with other beneficial bacteria, such as *Azospirillum* (Ribeiro et al., 2022) and *Bradyrhizobium* (Prando et al., 2020), to improve plant growth and increase the productivity of crops such as maize and soybean (Hungria et al., 2001; Abreu et al., 2017).

Although PGPB are widely recognized for enhancing plant growth, biomass accumulation, crop yield, as well as potentially increasing plant tolerance to herbivore attack, these benefits may indirectly favor phytophagous arthropods (Megali et al., 2015). For example, Megali et al. (2014) demonstrated that inoculating tomato plants

with beneficial microorganisms increased the survival rate of the pest *Spodoptera littoralis* by 25% and resulted in heavier caterpillars compared to those on non-inoculated plants. Such responses may be associated with increased nutrient availability and improved host plant quality following inoculation, conditions that may enhance herbivore fitness and population growth (Schoonhoven et al., 2005). Furthermore, inoculation can also alter the production of chemical defenses, thereby favoring herbivores (Pineda et al., 2013; Megali et al., 2014, 2015).

The incidence of the two-spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae) has increased considerably in soybean-growing areas, causing substantial yield losses (Ruffatto et al., 2024). This phytophagous mite feed on leaf cell contents, producing characteristic symptoms such as chlorosis, bronzing, and subsequent leaf abscission. These injuries directly impair photosynthetic efficiency, resulting in reduced biomass accumulation and lower grain filling (Scott et al., 2021).

Despite the well-documented agronomic benefits of PGPB, information regarding their effects on arthropod pests and the ecological consequences of these interactions remains limited. In this study, we evaluated the effects of soybean inoculation with *B. megaterium* (BRM 119), *B. subtilis* (BRM 2084), *Bradyrhizobium* sp., and *Azospirillum* sp., applied individually or in combination, on the mobility, host preference, and biological performance of the two-spotted spider mite.

Material and Methods

Spider mite rearing

Leaves of jack bean (*Canavalia ensiformis* (L.) DC) were used to maintain colonies of *T.urticae* in a climate-controlled chamber (BOD incubator) at 25 ± 1 °C, 70 ± 10% relative humidity, and a 12:12 h (L:D) photoperiod. The leaves were placed on water-saturated foam inside plastic trays with the abaxial surface facing upward. Leaf margins were surrounded with moistened cotton to prevent mite escape.

To obtain experimental individuals of uniform age, synchronized cohorts (“egg waves”) were established 15 days before the bioassays, following the methodology described by Helle (1967). Adult females of *T. urticae* were allowed to oviposit on the leaves for 24 h and were subsequently removed. Throughout the rearing period, moisture levels were monitored and maintained as required, and cohort development was followed for 15 days until all individuals reached adulthood.

Soybean plant inoculation

Three seeds of soybean (*Glycine max* L. cv. BRS Valiosa) were sown in 1.0-L plastic pots containing soil with the following physicochemical characteristics: pH (H_2O) = 6.1, organic matter = 19 g kg^{-1}, Mehlich-P = 7 mg kg^{-1}, Mehlich-K = 2.4 mmolc kg^{-1}, Ca = 24 mmolc kg^{-1}, Mg = 11 mmolc kg^{-1}, Al = 0 mmolc kg^{-1}, H + Al = 23 mmolc kg^{-1}, sum of bases = 37 mmolc kg^{-1}, cation exchange capacity = 60 mmolc kg^{-1}, base saturation = 62%, and aluminum saturation = 0%. Pots were maintained inside anti-aphid screen cages in a greenhouse.

Plants were grown in 24 pots arranged in a completely randomized design comprising six treatments with four replicates each. All treatments received 0.375 g of NPK fertilizer (10:10:10 and 1 mL of a micronutrient solution containing iron, manganese, zinc, copper, molybdenum, and boron.

The commercial bioinoculant BiomaPhos® containing strains BRM 119 (*B. megaterium*) and BRM 2084 (*B. subtilis*), both phosphate-solubilizing bacteria, was applied according to the manufacturer's recommendation (100 mL ha^{-1}). For each pot, 1 mL of the bacterial suspension was applied directly to the seeds at sowing.

A second treatment consisted of the application of BRM 119 (*B. megaterium*) and BRM 2084 (*B. subtilis*) combined with *Bradyrhizobium* inoculation. The bacterial strains were applied as described above, whereas *Bradyrhizobium* was included to simulate conditions commonly found under field cultivation (Hungria et al., 2015).

The third treatment consisted of inoculation with *Azospirillum* at a concentration of 10^9 colony-forming units (CFU). The fourth treatment involved co-inoculation of *Azospirillum* with strains BRM 119 (*B. megaterium*) and BRM 2084 (*B. subtilis*). The control treatment consisted of plants that received only the standard mineral fertilization (0.375 g). To distinguish the effects of plant growth-promoting bacteria from those resulting from increased nutrient availability, an additional treatment was included in which plants received double the NPK dose (0.750 g per plant) without bacterial inoculation. After seedling emergence, plants were thinned to one plant per pot. Irrigation and all other cultural practices were performed as required throughout the experiment.

Arena preference test with Tetranychus urticae

Soybean plants from each treatment were grown in a greenhouse until reaching the V3 developmental stage. Pots corresponding to the six treatments were arranged in a circular arena (90 cm internal diameter), with a distance of 10 cm between adjacent pots (Fig. 1).

A total of 100 adult individuals were collected from spider-mite colonies and released at the center of the arena. The experiment was replicated four times. In each replicate, the position of the pots (treatments) was randomly changed to minimize potential effects of external factors, such as light incidence or air movement. Mites were allowed to select a host plant for 24 h. After this period, the number of *T. urticae* individuals recaptured on each soybean plant was recorded following the methodology described by Hata et al. (2020).

Physiological parameters

Analysis of Fresh Biomass, chlorophyll content, leaf area, and root length measurements

Soybean plants from each of two treatments were weighed on an analytical scale to obtain fresh weight. Chlorophyll content was determined using a chlorophyll content meter (CCM-200 Plus, Opti-Sciences Inc., Hudson, NH, USA). The instrument operates based on the absorbance of light emitted by diodes at three wavelengths, 635 and 660 nm (red region) and 940 nm (near-infrared region), allowing non-destructive estimation of leaf chlorophyll content. Measurements were performed on fully expanded leaves of soybean plants, and chlorophyll content was expressed as chlorophyll content index (CCI) units, according to the manufacturer's specifications.

Additionally, the leaf area index (LAI) was measured in three replicates per treatment using photographs taken with a ruler included as a scale reference. The images were analyzed using the image-processing algorithm implemented in the ImageJ software (McDonald et al, 2022).

When the soybean plants reached the V7 vegetative stage, plants from all treatments were removed from their pots for root evaluation. The shoots were separated from the root system and taken to the laboratory to determine fresh biomass. The roots were carefully removed from the pots, gently shaken to remove excess soil, and then washed under running water. Each root system was placed in a container containing 50 mL of 70% ethanol for subsequent analysis using the WinRHIZO image analysis system (Regent Instruments Inc., Quebec, Canada). The root parameters evaluated were total root length (RL) and root volume (RV) (Arsenault et al., 1995).

Nitrogen and phosphorus analyses

Soybean plants at the V7 vegetative stage were used for nitrogen (N) and phosphorus (P) analyses. Leaf phosphorus concentration was determined following tissue extraction using a modified Mehlich-1 double-acid extraction procedure based on the method described by Mehlich (1953). The extracting solution consisted of 0.05 mol L^{-1} hydrochloric acid (HCl) and 0.0125 mol L^{-1} sulfuric acid (H_2SO_4), which promotes the solubilization of available and weakly adsorbed phosphorus forms present in plant tissues.

Leaf samples were washed with distilled water, dried in a forced-air oven at 65 °C until constant weight, and ground in a Wiley mill fitted with a 1-mm screen. For extraction, 0.5 g of dried tissue was mixed with 50 mL of Mehlich-1 solution. The suspension was shaken on a horizontal shaker at 200 rpm for 5 min and subsequently filtered through qualitative filter paper.

Phosphorus concentration in the filtrate was determined spectrophotometrically using the molybdenum blue method. In this procedure, phosphorus reacts with a reagent containing ascorbic acid, ammonium molybdate, and sulfuric acid, producing a blue-colored complex whose intensity is proportional to the phosphorus concentration in the sample. Absorbance was measured at 725 nm.

For nitrogen determination, leaf samples were dried at 65 °C and subjected to sulfuric digestion using an H_2SO_4–H_2O_2 mixture. Total nitrogen concentration was quantified using the Kjeldahl method according to Morais and Rabelo (1986) and Cheng et al. (2022). Nitrogen accumulation in leaves was calculated by multiplying leaf dry mass by the corresponding nitrogen concentration in plant tissue. The resulting data were analyzed using regression analysis.

Development, survival, and oviposition assays

To determine the biological parameters of *T. urticae*, leaf discs (5 cm in diameter) were excised from the central portion of soybean leaves collected from the six treatments described above. Leaf discs were placed individually in 35-mm-diameter plastic Petri dishes lined with a thin layer of moistened cotton, with the abaxial leaf surface facing upward. Subsequently, a one-day-old Tetranychus urticae egg was carefully transferred to the center of each leaf disc using a fine paintbrush. To ensure that all eggs were of the same age at the beginning of the experiment, 40 ovipositing females were isolated 24 h before the experiment and maintained on jack bean leaves to obtain eggs of same age. The leaf discs were replaced with fresh ones every two days. Juvenile development was monitored daily until individuals either reached adulthood or died. Fifteen replicates were established for each treatment

For the oviposition assay, females that were two days old after adult emergence were used. Each female was individually transferred to a leaf disc from one of the treatments described above. Oviposition was recorded every 24 h for four consecutive days. Fifteen replicates were established for each treatment.

Population dynamics of *Tetranychus urticae*

Soybean plants at the V3 phenological stage were infested with five adult females of *T. urticae* placed on the abaxial surface of the youngest fully expanded trifoliate leaf. The infested leaflets were isolated with entomological adhesive, allowing easier monitoring throughout the experiment while preventing mite dispersal to other leaves.

The density of mobile mite stages on soybean leaves was monitored through six consecutive evaluations conducted at 3-day intervals, covering a total period of 19 days after infestation. During each assessment, the number of mobile *T. urticae* individuals present on the plants was recorded. At the final evaluation (19 days after infestation), a destructive assessment was conducted. All leaves were detached from the plants and examined under a stereomicroscope to accurately quantify the total number of remaining mobile mites.

Statistical analyses

Data on the number of mobile *Tetranychus urticae* individuals recorded in each treatment were analyzed using a completely randomized design. Count data were analyzed using generalized linear models (GLMs) with a Poisson error distribution and log-link function (Crawley 2013). When significant treatment effects were detected, means were compared using Tukey's post hoc test implemented in the *emmeans* package (Lenth 2016).

For the arena preference assay, the number of *T. urticae* individuals recaptured on soybean plants was analyzed using a completely randomized design. Prior to model fitting, residual normality and homogeneity of variances were assessed using the Shapiro–Wilk and Levene tests, respectively, at a significance level of 5%. Potential outliers were identified through visual inspection of boxplots. Subsequently, generalized linear models (GLMs) with a negative binomial error distribution were fitted because this distribution is more appropriate for overdispersed count data (Crawley 2013). The response variable was the number of adult female *T. urticae* recaptured on soybean plants, whereas seed treatment with bioinoculants was included as the explanatory variable. Model fit was evaluated using the *HNP* package, and multiple comparisons among treatments were performed using the *multcomp* package.

Plant physiological parameters were analyzed by analysis of variance (ANOVA), and treatment means were compared using Tukey's test at the 5% significance level. The effects of the bioinoculants on the survival and juvenile developmental time of *T. urticae* were analyzed using a Cox proportional hazards survival model implemented in the survival package in R (Therneau, 2023). Pairwise comparisons among treatments were performed by aggregating treatment levels.

The effects of the bioinoculants on oviposition rate were analyzed using a linear mixed-effects model, with treatment and time as fixed factors and individual mites as a random factor to account for repeated measurements (function lme, nlme package; Pinheiro et al., 2020). To eliminate potential carry-over effects from the previous diet, data collected on the first day of oviposition were excluded from the analysis (Sabelis,

1990). Pairwise comparisons among treatments were performed using the emmeans package with Tukey's adjustment for multiple comparisons (Lenth, 2019).All statistical analyses, model fitting procedures, and graphical outputs were performed in R version 4.4.1 (R Core Team 2024). The packages *MASS*, *car*, and *rstatix* were used for statistical analyses, whereas *ggplot2* was used for data visualization. Numerical datasets, analysis scripts, and graph-generation codes were deposited in a publicly accessible GitHub repository to promote transparency, reproducibility, and independent verification of the results (Gandrud 2020)

Results

Preference test

There was a significant effect of soybean inoculation with bioinoculants on the host preference of *T. urticae* (GLM, negative binomial distribution, $\chi^2 = 19.31$, df = 5, $P = 0.001$). In choice arenas, we recuperated a greater number of mites on plants inoculated with *B. subtilis + B. megaterium*, *B. subtilis + B. megaterium + Azospirillum*, *B. subtilis + B. megaterium + Bradyrhizobium*, or *Azospirillum* compared with non-inoculated plants (Fig. 2).

Physiological parameters

Soybean plants receiving fertilizer or soil-applied bioinoculants exhibited greater fresh weight than non-treated plants (ANOVA, F = 3.07, df = 18, $P = 0.03$; Fig. 3). These treated plants also showed higher nitrogen and phosphorus contents than the control group. However, parameters such as leaf area, chlorophyll content, and root length did not consistently increase across treatments when compared with the control (Table 1).

Table 1
Foliar contents of nitrogen (N) and phosphorus (P), leaf area, chlorophyll content, and root length of soybean plants that received soil bioinoculants compared with the control. Means followed by the same letter do not differ significantly according to Tukey's test at the 5% significance level, as obtained using the *emmeans* package.

Treatment	N		P		Leaf area (cm^2)		chlorophyll		Root length (mm)	
Control	2.8	a	1178.9	a	145.3	b	5.8	c	2533.5	a
NPK	3.6	b	1846.8	c	150.2	ab	10.4	a	2579.0	a
B. subtilis + B. megaterium	3.5	b	1410.9	b	164.4	a	10.2	ab	3067.6	d
B. subtilis + B. megaterium +Azospirillum	3.7	b	1464.2	b	102.3	c	9.8	ab	1785.9	b
B. subtilis + B. megaterium +Bradyrhizobium	3.3	ab	2119.8	d	157.6	ab	9.2	b	2567.6	a
Azospirillum	3.7	b	1853.4	c	65.2	d	10.8	a	1489.4	c

Development, survival, and oviposition assays

Soybean inoculation affected the juvenile developmental time of *T. urticae* (Fig. 4; ANOVA, F = 4.07, df = 82, $P < 0.01$). Juvenile feeding on plants inoculated with *Bacillus subtilis + B. megaterium* reached adulthood more rapidly than those in the other treatments, whereas individuals that fed plants inoculated with *B. subtilis + B. megaterium + Bradyrhizobium* required the longest time to complete development. Developmental times in the remaining treatments were intermediate (Fig. 4).

There was a significant effect of soybean fertilization and soil inoculation (fixed effect) on oviposition rate of *Tetranychus urticae* (Fig. 5; GLMM, poisson distribution, $\chi^2 = 75.89$, df = 5, $P < 0.001$). Time (days) (random effect), as expected, also positively affected the oviposition of *T. urticae* (GLMM, poisson distribution, $\chi^2 = 21.70$, df = 1, $P < 0.001$). Mites feeding on plants treated with *B. subtilis + B. megaterium*, *B. subtilis + B. megaterium + Bradyrhizobium*, NPK, or *B. subtilis + B. megaterium + Azospirillum* laid more eggs than those feeding on control plants. The lowest oviposition was recorded in the control treatment (Fig. 5).

Population dynamics of Tetranychus urticae

Soybean fertilization and bioinoculation (fixed effect) significantly affected the abundance of *Tetranychus urticae* mobile stages (GLMM, Poisson distribution, $\chi^2 = 21.88$, df = 5, $P < 0.001$). Time (days) (random effect), as expected, also positively affected the oviposition of T. urticae (GLMM, poisson distribution, $\chi^2 = 945.58$, df = 1, $P < 0.001$). The highest numbers of mobile stages were recorded on plants treated with NPK, *B. subtilis + B. megaterium + Azospirillum*, or *Azospirillum*, whereas the control treatment supported the lowest population densities (Fig. 6).

Discussion

Bioinoculants containing *Bacillus megaterium* and *B. subtilis* significantly improved soybean physiology, as evidenced by increased chlorophyll content, photosynthetic efficiency, and fresh biomass. However, these changes also enhanced the performance of *Tetranychus urticae*, resulting in greater longevity, fecundity, and developmental rate of the mite. These findings suggest that bioinoculation may intensify herbivory through indirect plant-mediated effects. Although bioinoculants represent a promising tool for promoting plant growth, their use should be integrated with pest management strategies that take into account their potential beneficial effects on herbivores. Our results indicate that bioinoculation may increase both the preference and performance of *T. urticae*, highlighting the need for careful evaluation of its adoption in production systems where this mite is a key pest.

Understanding the interactions between plant growth-promoting microorganisms and phytophagous arthropods has advanced considerably within the framework of integrated pest management, particularly regarding indirect effects mediated by the host plant. Our results showed that the greater recapture of mobile individuals on inoculated plants, compared with the control, indicates that inoculation increased the plant's carrying capacity for the herbivore. This pattern is consistent with the findings of Khaitov et al. (2015), who reported an increase in the reproductive rate of *T. urticae* on plants inoculated with *Azotobacter chroococcum* and *Glomus mosseae*, as well as a preference for inoculated plants in choice assays. According to these authors, the increased availability of nutrients and greater plant biomass contribute to improved host quality. This hypothesis is further supported by the present study, in which inoculated plants exhibited increased fresh biomass.

The increase in *T. urticae* densities observed in treatments containing *Azospirillum* may be explained by increased chlorophyll content in plants inoculated with this bacterial genus (Marques et al., 2023). Higher chlorophyll levels are directly associated with enhanced photosynthetic efficiency and, consequently, greater production of photoassimilates, leading to improved plant nutritional quality. Similar findings were also reported by Oliveira et al. (2022), who observed increases in chlorophyll index and root biomass in plants inoculated with *A. brasilense*. These effects indicate an intensification of plant metabolism, possibly associated with enhanced nutrient assimilation and hormonal regulation, contributing to the development of plant tissues that are more suitable for herbivore feeding and development.

Although inoculation with plant growth-promoting bacteria may enhance plant tolerance to herbivore attack (Rashid et al. 2017), the results of the present study indicate that this effect did not translate into a reduction in *T. urticae* densities. From a pest management perspective, such responses may be considered undesirable, as they favor population growth of this mite. Therefore, the adoption of bioinoculants should take into account potential indirect plant-mediated effects that may compromise the balance between promoting plant growth and regulating arthropod pest populations. The increase in fresh biomass indicates a higher water content in plant tissues. Given that *T. urticae* feeds on cellular contents, more turgid tissues likely enhance feeding efficiency, leading to improved biological performance and, consequently, greater mite abundance.

These findings contrast with those reported by Hosseini et al. (2022), who observed reduced development of *T. urticae* on strawberry plants inoculated with *Azotobacter chroococcum*, *A. brasilense*, and *Pseudomonas brassicacearum*. Similarly, Pappas et al. (2021) reported decreased mite oviposition and aphid population growth in inoculated plants, attributing these effects to increased production of secondary metabolites. The role of host quality is further supported by Li et al. (2002), who demonstrated that plants of lower quality led to reduced oviposition rates. In contrast, in the present study, the increased oviposition rate observed in some treatments, particularly with the inoculant (*Bacillus megaterium* + *Bacillus subtilis*), indicates an improvement in plant nutritional quality.

Physiological assessments further support this pattern, as plants treated with *B. subtilis* + *B. megaterium* exhibited higher chlorophyll indices, indicating enhanced photosynthetic efficiency and improved light-use capacity. These findings are consistent with the reported modes of action of *B. subtilis*, which include stimulation of root growth, increased nutrient availability, and the accumulation of metabolites that promote plant development (Carrégalo-Ríos et al., 2025).

In conclusion, inoculation with *B. megaterium* and *B. subtilis* markedly enhanced soybean physiological performance, as reflected by increased chlorophyll content and fresh biomass, however, these benefits were accompanied by a concomitant increase in the performance of *T. urticae*, including higher longevity, fecundity, and accelerated development. This pattern demonstrates that bioinoculation can inadvertently increase plant susceptibility to herbivory through plant-mediated effects on host quality. Consequently, although these microorganisms represent valuable tools for promoting plant growth, their use must be critically evaluated within an integrated pest management framework, particularly in systems where *T. urticae* is a key pest, to avoid unintended amplification of herbivore populations.

Declarations

Competing interests

The authors declare no competing interests.

Ethics approval

This article does not contain any studies with human participants or vertebrate animals performed by any of the authors.

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Author Contribution

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by [Dalila Dominique Duarte Rocha], [Ivanildo Evódio Marriel] and [Marcos Antonio. Matiello. Fadini]. The first draft of the manuscript was written by [Dalila Dominique Duarte Rocha], [Priscilla Tavares Nascimento Maia], [Ítalo dos Santos Faria Marcossi], [Marcos Antonio. Matiello. Fadini] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data Availability

The data have been archived on the GITHUNB Repository at: https://github.com/marcosfadini/Tetranychus-urticae-prefers-soybean-plants-with-higher-nutritional-quality

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Figures

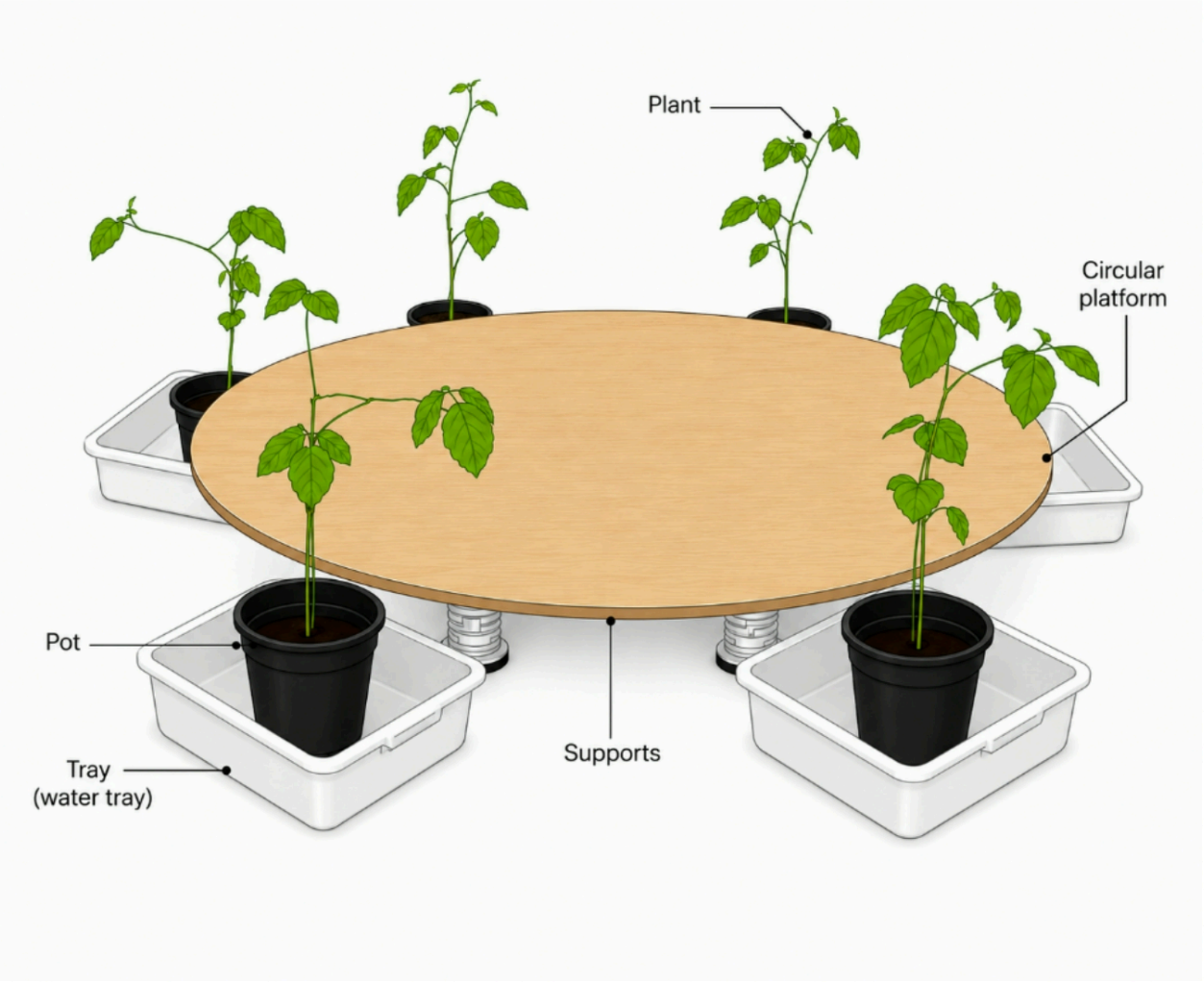


Figure 1

Foraging arena used to evaluate the behavior of adult female *Tetranychus urticae* under laboratory conditions. Six plants, corresponding to the evaluated treatments, were arranged equidistantly in a circular pattern around the arena. One hundred adult females were released at the center of the arena in each of the four independent replicates. After each replicate, plants were replaced, the arena was cleaned, and treatment positions were randomly reassigned to minimize potential environmental effects on mite distribution.

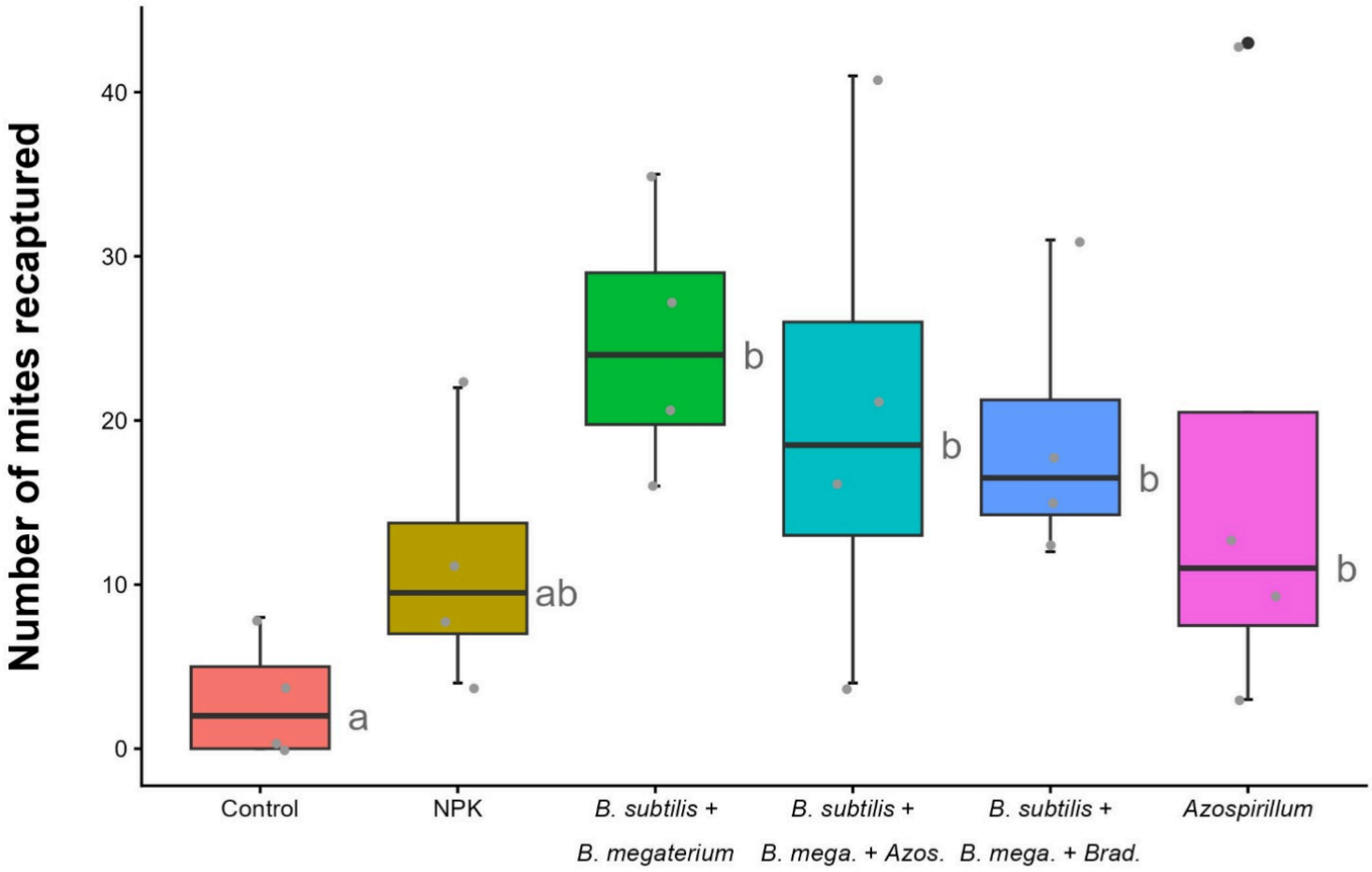


Figure 2

Box plot of the number of adult females of *Tetranychus urticae*recaptured on soybean plants treated with soil bioinoculants compared with the control in arena choice tests. Gray dots around the boxes represent the observed values of the response variable in the experiment. Boxes followed by the same letter do not differ significantly at the 5% significance level according to the analysis performed using the *multcomp*package.

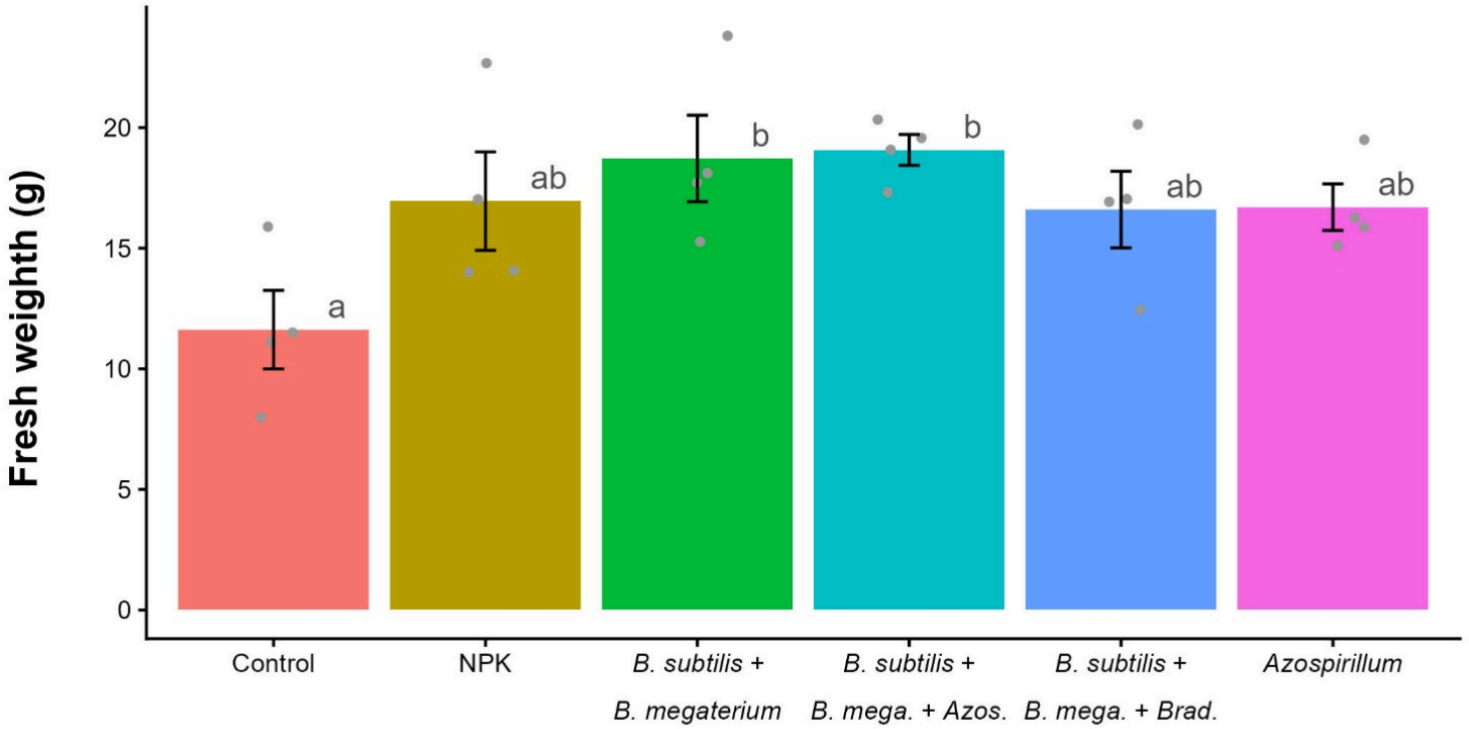


Figure 3

Mean fresh weight (± standard error) of soybean plants treated with soil bioinoculants compared with the control. Gray dots around the boxes represent the observed values of the response variable in the experiment. Bars followed by the same letter do not differ significantly according to Tukey's test at the 5% significance level, as obtained using the *emmeans* package.

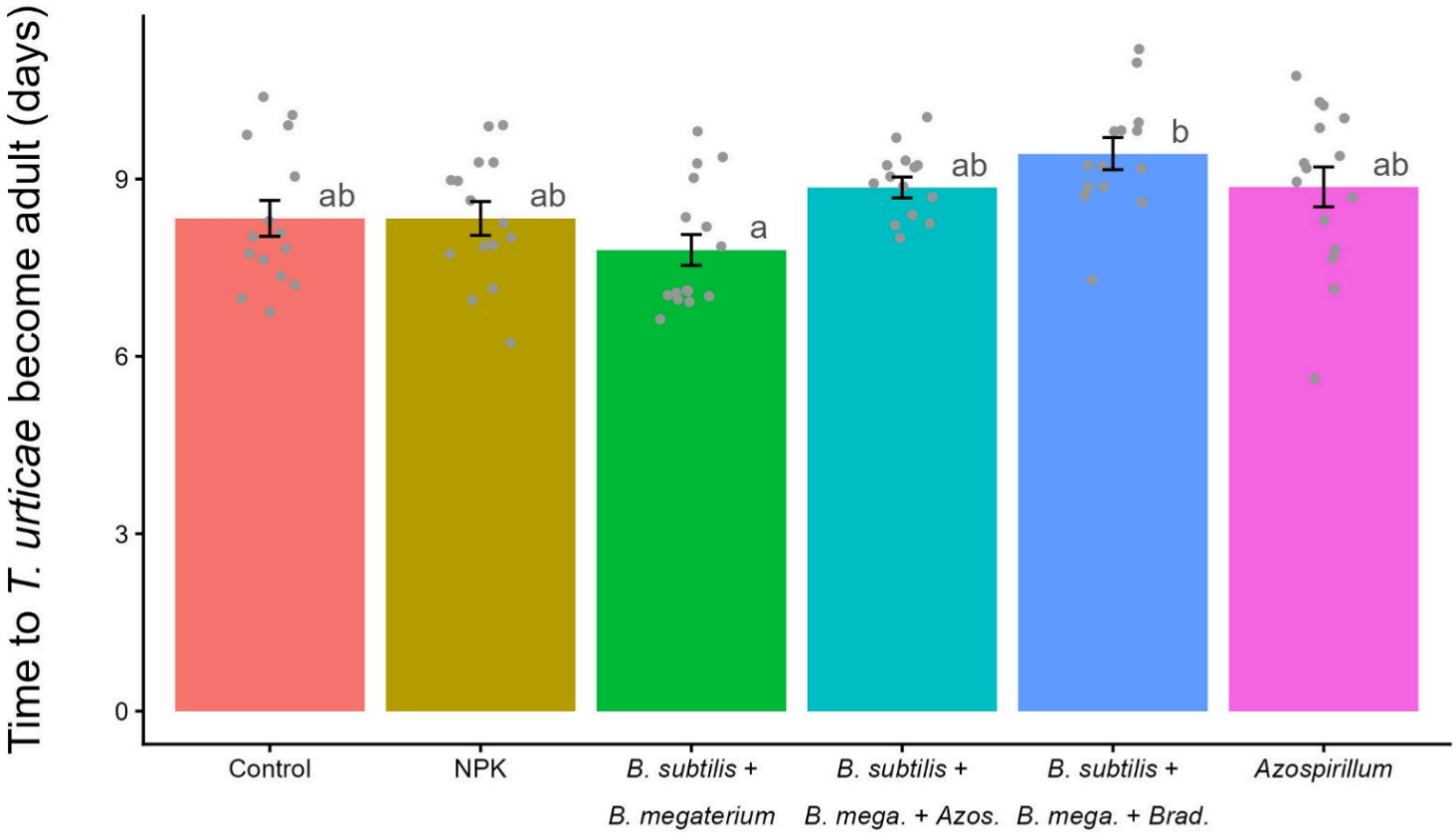


Figure 4

Mean time (± standard error), in days, required for the two-spotted spider mite *Tetranychus urticae* to reach adulthood on soybean plants treated with soil bioinoculants compared with the control. Gray dots around the boxes represent the observed values of the response variable in the experiment. Bars followed by the same letter do not differ significantly according to Tukey's test at the 5% significance level, as obtained using the *emmeans* package.

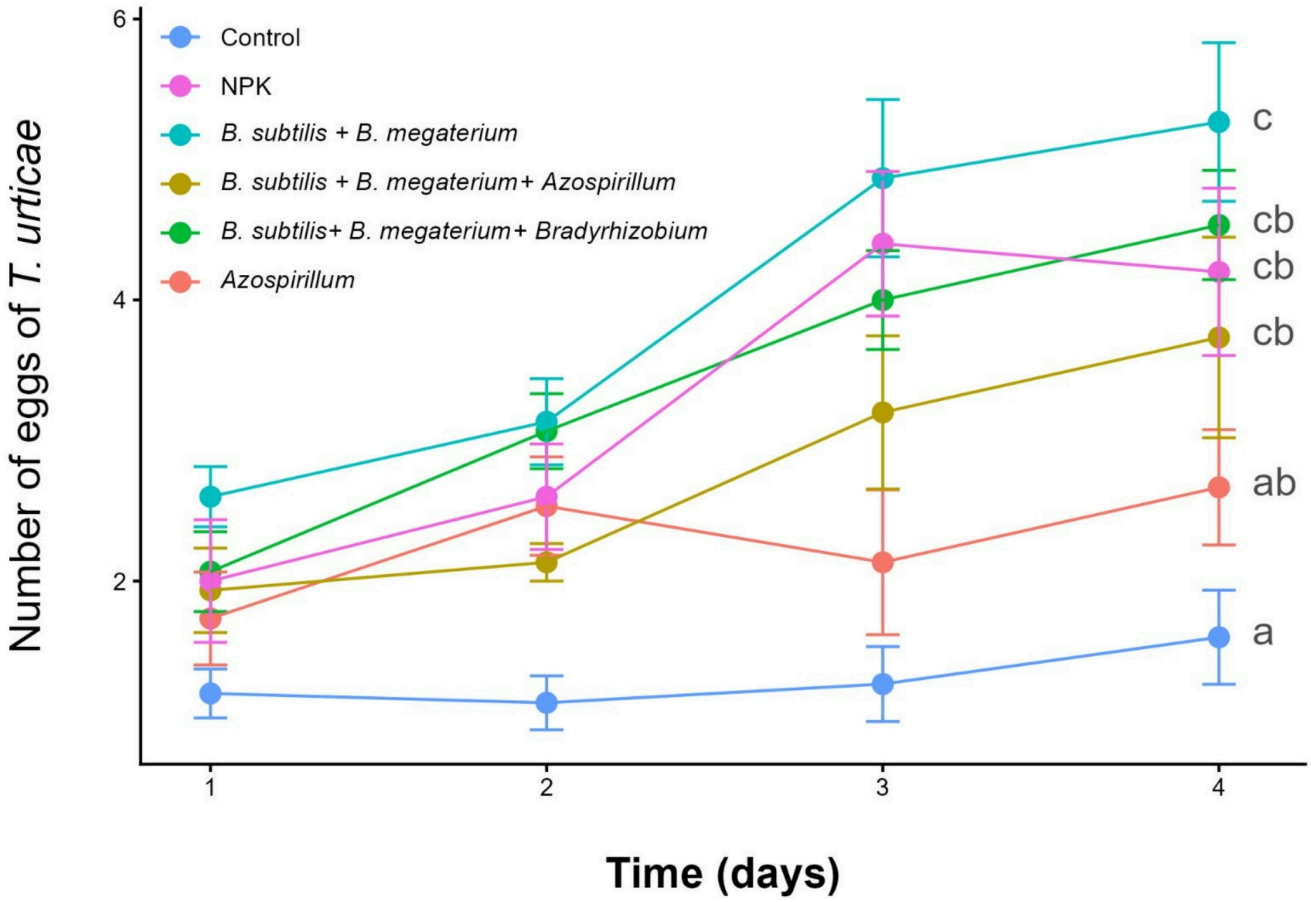


Figure 5

Mean number (± standard error) of eggs laid by the two-spotted spider mite *Tetranychus urticae* on soybean plants treated with soil bioinoculants compared with the control over time (days). Lines followed by the same letter do not differ significantly according to Tukey's test at the 5% significance level, as obtained using the *emmeans* package.

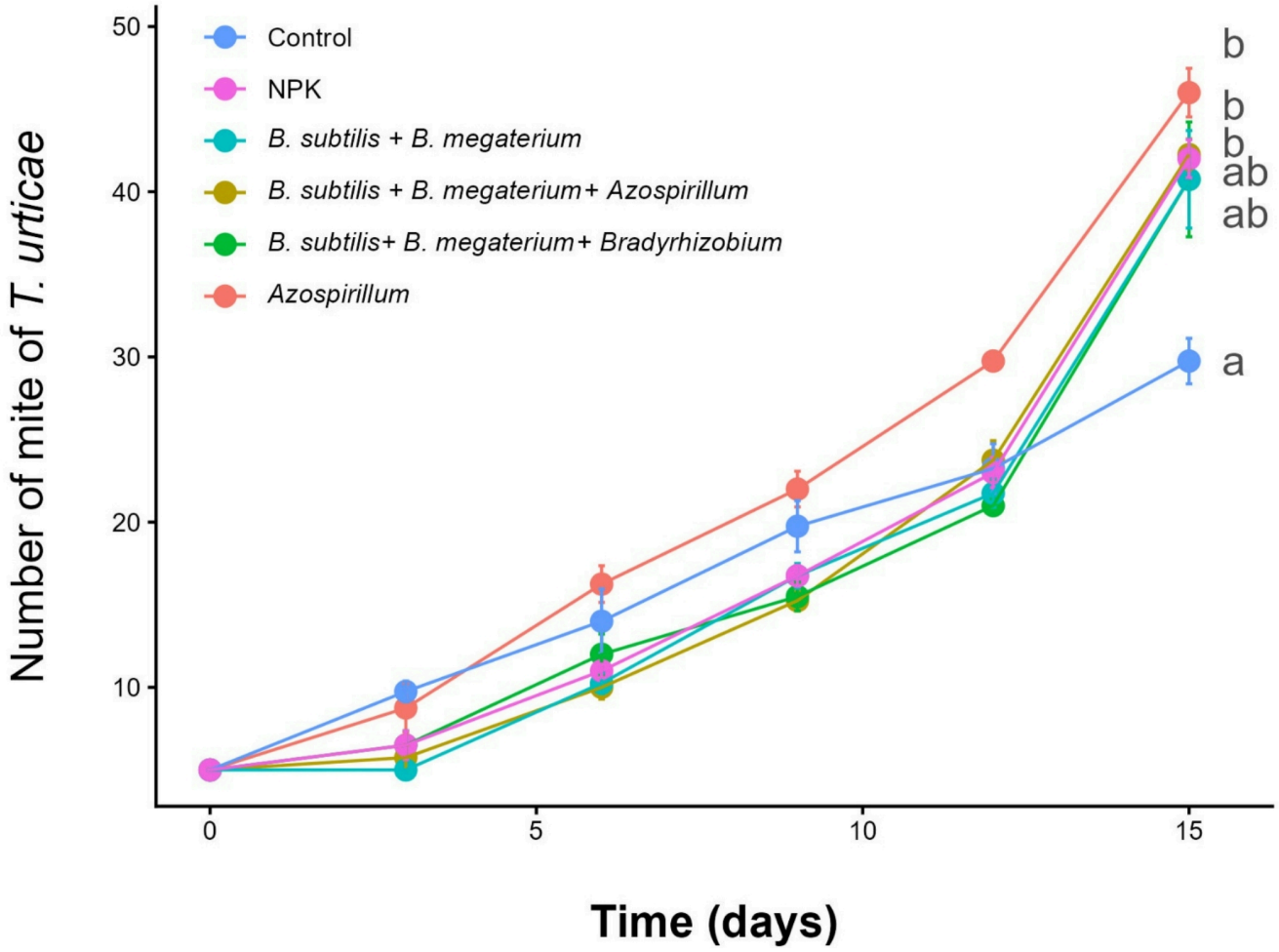


Figure 6

Mean number (± standard error) of mobile forms of the two-spotted spider mite *Tetranychus urticae* on soybean plants treated with soil bioinoculants compared with the control. Gray dots around the boxes represent the observed values of the response variable in the experiment. Bars followed by the same letter do not differ significantly according to Tukey's test at the 5% significance level, as obtained using the *emmeans*package.