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## COMPARATIVE FIELD EVALUATION OF A NATIVE *BEAUVERIA BASSIANA* STRAIN AND THE COMMERCIAL PRODUCT NATURALIS® FOR THE CONTROL OF *MACROSIPHUM ROSAE*

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### Abstract

The use of entomopathogenic fungi as biological control agents is increasingly recognized as an essential component of sustainable pest management. Unlike conventional insecticides, these fungi are environmentally safe, non-toxic to humans, and less likely to induce pest resistance. This study evaluated the efficacy of *Beauveria bassiana* against the green rose aphid (*Macrosiphum rosae* Linnaeus, 1758) in an organic Damask rose plantation in Kliment village, Bulgaria, during 2024–2025. The experiment followed a randomized complete block design with three replications and 18 m<sup>2</sup> plots. Two fungal products were compared: the commercial formulation Naturalis® (*B. bassiana* ATCC 74040, 0.185 g kg<sup>-1</sup>) at 100 ml da<sup>-1</sup> and a native *B. bassiana* strain 214 (GenBank OM366244) at 300 ml da<sup>-1</sup>, along with an untreated control. Both products were applied twice at 10-day intervals. Naturalis® achieved the highest efficacy, reducing aphid populations by up to 83% following double application, whereas strain 214 provided moderate control ranging from 29% to 48%, depending on the year and treatment frequency. Aphid population dynamics were strongly influenced by climatic conditions, particularly rainfall and temperature. These findings confirm that *B. bassiana*-based products, especially Naturalis®, provide effective and environmentally friendly control of *M. rosae* in organic rose cultivation. Although less efficient, the native strain 214 shows potential as a locally adapted biocontrol agent. Further optimization of formulation, dosage, and application timing is recommended to enhance its contribution to integrated pest management in rose production systems.

**Keywords:** *Beauveria bassiana*, Damask rose, green rose aphid, organic pest management

### INTRODUCTION

During the growing season, the oil-bearing rose can be attacked by various pests, which are capable not only of slowing growth but also of compromising the quality of the essential oil (Golizadeh et al., 2017). The results of the study by Yilmaz (2015) show that in Turkey, aphids are the most common sucking insects in rose

plantations, with a frequency of about 77%. Demirözer et al. (2011) estimate that the green rose aphid (*Macrosiphum rosae* L.) and *Rhodococcus perrornatus* Cockerell & Parrott, are the most economically important pests recorded in oil-bearing rose plantations in Isparta, Turkey. In addition to causing deformation of leaves and lower stalks, aphids serve as vectors for various viruses (Chau & Heinz, 2004). In some cases, the attack by these pests can cause changes in the biochemical processes of plants. As a result of the sucking activity of insects, the quality of the produce deteriorates, plants weaken and become susceptible to disease attacks (Parker et al., 2013). In every farm, it is necessary to develop a successful strategy based on a plan for implementing integrated pest management and regular monitoring of pest population density (James, 2003). Aphids multiply at their highest density during the spring-summer period, when they cover (in a colony) the entire growing tip of the plant. Their reproduction rate is extremely fast. The main types of aphids found in rose plantations are the green rose aphid, cotton aphid (*Aphis gossypii* Glover), and green peach aphid (*Myzus persicae* Sulzer), which can coexist and reproduce simultaneously on the oil-bearing rose (Hegde et al., 2020). Of all those listed, the green rose aphid (*Macrosiphum rosae*) is the most widespread and dominant (Hegde et al., 2020). Female aphids give birth to young nymphs, which are capable of becoming mothers within a week to just a few days. Typically, the population contains more female individuals, which can reproduce through parthenogenesis. Thanks to the high reproductive rate under favourable conditions, aphids can quickly multiply and form large populations.

Controlling sucking insects can be challenging due to their developed pesticide resistance, rapid development rates, and high fecundity potential (Norboo et al., 2017). In nature, there are many natural predators, parasites, and diseases that help control aphid populations. The main factor that can negatively affect the development of these pests is unfavourable climatic conditions. In limited areas, heavy spraying of the populations with a water jet for physical removal can be practised as an alternative control method, but in large production areas, the control is carried out using conventional or biological plant protection products (Hegde et al., 2020). Pest control can be successfully achieved biologically through organic plant protection products or by introducing biological agents. Entomopathogenic fungi are important natural regulators of insect populations and play a key role in maintaining ecological balance in terrestrial ecosystems. They infect a wide range of hosts and have been extensively studied for their potential in integrated pest management programs (Hajek & Leger, 1994; Goettel & Inglis, 1997). These fungi, primarily belonging to the genera *Beauveria*, *Metarhizium*, *Isaria*, and *Lecanicillium*, are among the most widely applied microbial control agents in agriculture (Inglis et al., 2001). Compared to conventional insecticides, entomopathogenic fungi offer several advantages. They are inexpensive, easy to apply, highly effective, and harmless to humans and the ecosystem (Lacey et al., 2001), which has led to the commercialisation of a large number of new biopesticide products based on entomopathogenic fungi (Faria & Wraight, 2001). Several sources report the high potential of *Lecanicillium lecanii* (Zimmerman) and *Beauveria bassiana* (Balsamo) in the biological control of aphids (Askary et al., 1998; Derakhshan et al., 2007; Abd El-Salam & El-Hawary, 2011). There is a lack of sufficient information on feeding

behaviour, population structure, factors affecting distribution, and variations related to the virulence of *Beauveria bassiana* (Chandler, 2005). To date, large-scale scientific studies on the potential of *B. bassiana* have focused on two plant species, maize (*Zea mays*, 43% of studies) and faba bean (*Vicia faba*, 34% of studies), and almost half of the research has targeted a single pest group—the maize stem borer (McKinnon et al., 2017). The literature review highlights the need for further studies, including long-term field experiments, different crops, and a broader range of insect pests, to evaluate the potential of this biocontrol agent.

This study aimed to evaluate the efficacy of a native *Beauveria bassiana* strain (214, GenBank OM366244) compared with the commercial formulation Naturalis® (*B. bassiana* ATCC 74040) for the biological control of the green rose aphid (*Macrosiphum rosae* L.) under field conditions in organic rose plantations. The research sought to determine how product type and application frequency (single versus double treatment) influence aphid population dynamics and control efficacy, and to assess the potential of the native isolate as a locally adapted bioinsecticidal agent for sustainable pest management in rose cultivation.

## MATERIAL AND METHODS

### **Weather Conditions**

Meteorological data were collected from an automatic weather station installed within the experimental field at Kliment village (Plovdiv region, Bulgaria). The station continuously recorded daily air temperature (°C) and precipitation (mm) throughout the study period (2024–2025). Mean monthly temperature and total precipitation were calculated for both years to characterize climatic variation during the growing seasons.

### **Experimental site and plant material**

The field experiment was conducted during the 2024–2025 growing seasons in a certified organic plantation of *Rosa damascena* Mill. located near Kliment village, Plovdiv region, Bulgaria (42°33' N, 24°45' E). The plantation consisted of five-year-old rose plants grown under standard agroecological management without chemical pesticides or synthetic fertilizers. The soil was classified as loamy Cambisol with a pH of 6.8 and organic matter content of 2.4%.

### **Experimental design and treatments**

A randomized complete block design with three replications was employed. Each experimental plot measured 18 m<sup>2</sup> and contained 21 rose plants. Rows were spaced 3.0 m apart, with 0.4 m between plants within a row.

### **Treatments evaluation**

**Naturalis®** – a commercial bioinsecticide containing *Beauveria bassiana* strain ATCC 74040 (0.185 g kg<sup>-1</sup> active ingredient), applied at a rate of **100 mL da<sup>-1</sup>**. ***Beauveria bassiana* strain 214** – a locally isolated entomopathogenic strain (GenBank accession no. OM366244), prepared as a water-based spore suspension at **300 mL da<sup>-1</sup>**. **Untreated control** – received water only. Foliar applications were performed twice at 10-day intervals during April and May of each year, coinciding with the early vegetative and pre-flowering stages. The spray volume was

standardized to 400 L ha<sup>-1</sup> using a manual backpack sprayer. The treatments were carried out in the early morning to minimize photoinactivation and desiccation of conidia.

#### **Monitoring of aphid populations**

The green rose aphid (*Macrosiphum rosae* L.) was selected as the target pest due to its high abundance and economic impact in the region. Aphid population density was determined following the washing method of Boller (1984). From each plot, 20 rose leaves and petals were randomly collected and rinsed with distilled water through a 63 µm sieve to separate aphids from plant debris. The total number of live insects was counted under a stereomicroscope (25× magnification). Sampling was performed before treatment and at 3, 5, and 7 days after each application (DAT).

#### **Assessment of treatment efficacy**

The efficacy of each treatment was calculated using the **Henderson–Tilton formula** (Lambrev, 2010):

$$E(\%) = [1 - ((N_t / N_{t_0}) / (N_c / N_{c_0}))] \times 100$$

where  $N_t$  and  $N_{t_0}$  represent the mean number of live aphids in the treated plots after and before application, and  $N_c$  and  $N_{c_0}$  represent the corresponding values in the untreated control.

#### **Statistical analysis**

All data were subjected to analysis of variance (**ANOVA**) using the general linear model for a factorial experiment with the following fixed factors: product (*B. bassiana* strain or Naturalis®), application frequency (single or double), and observation period (DAT = 3, 5, 7). Year was treated as a random factor. Mean comparisons were performed using Tukey's HSD test at  $p < 0.05$ . Boxplots and interaction plots were generated to visualize treatment effects using **R version 4.3** (R Foundation for Statistical Computing, Vienna, Austria).

## **RESULTS AND DISCUSSION**

#### **Weather conditions**

Data from the weather station recorded temperature and precipitation values, which are presented in Table 1. The climatic data indicate that there is an established trend of increasing average monthly temperatures in the area, although the values in both years are below the climatic norm. The annual rainfall in the first year is 895.5 mm, which is 35.9 mm below the annual norm of the region. In the second year, the same pattern is observed.

The climatic data indicated that the region is characterized by a **temperate continental climate** with warm summers and mild winters. During the experimental period, the mean monthly temperatures ranged from **4.1 °C in February to 28.0 °C in August 2024**, and from **4.1 °C in February to 26.2 °C in August 2025** (Table 1). Precipitation was unevenly distributed, with higher rainfall in May and early summer, followed by a drier period in July and August.

**Table 1.** Mean monthly temperature and precipitation during the 2024–2025 study period.

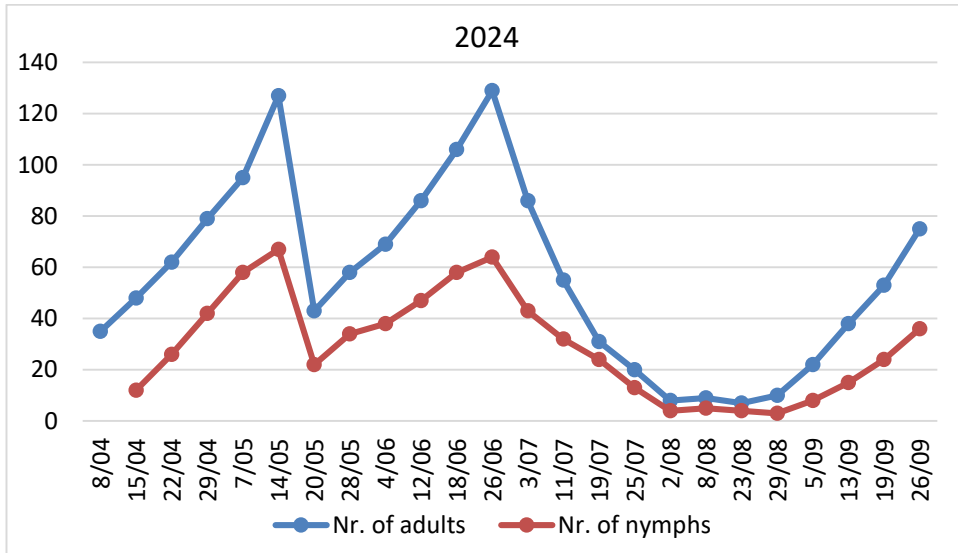
| Year          | Average monthly temperature (°C)   |      |      |      |       |      |      |      |       |      |      |      |
|---------------|------------------------------------|------|------|------|-------|------|------|------|-------|------|------|------|
|               | I                                  | II   | III  | IV   | V     | VI   | VII  | VIII | IX    | X    | XI   | XII  |
| 2024          | 4.4                                | 9.4  | 13.4 | 19   | 22.5  | 25.3 | 27.2 | 28.0 | 22.3  | 16.4 | 14.2 | 5.1  |
| 2025          | 4.1                                | 8.7  | 10.2 | 12.6 | 16.9  | 23.8 | 24.9 | 26.2 | 25.2  | -    | -    | -    |
| climatic norm | 0.5                                | 2.2  | 5.1  | 10   | 16.5  | 20.8 | 24.5 | 24.5 | 17.4  | 14.3 | 8.5  | 2    |
| Year          | Average monthly precipitation (mm) |      |      |      |       |      |      |      |       |      |      |      |
|               | I                                  | II   | III  | IV   | V     | VI   | VII  | VIII | IX    | X    | XI   | XII  |
| 2024          | 60.6                               | 68   | 73.2 | 80   | 120.4 | 54.2 | 41   | 60.4 | 78.7  | 82   | 86.6 | 90.4 |
| 2025          | 58.2                               | 44.5 | 70   | 51   | 70.2  | 52.7 | 33.2 | 48.4 | 51.8  | -    | -    | -    |
| climatic norm | 70                                 | 80   | 72.8 | 72   | 110.4 | 50.5 | 40   | 50.5 | 100.2 | 80   | 115  | 90   |

**Population dynamics of the green rose aphid**

The seasonal population dynamics of *Macrosiphum rosae* followed the phenological development of *Rosa damascena* during both experimental years. The first aphid colonies appeared in early April and reached their highest abundance in May, coinciding with the period of intensive shoot and leaf growth. Population density decreased sharply at the beginning of summer when average daily temperatures exceeded 22°C, consistent with previous findings by Dara (2017) and Mehrparvar et al. (2016), who reported reduced aphid reproduction under high temperature and low humidity conditions.

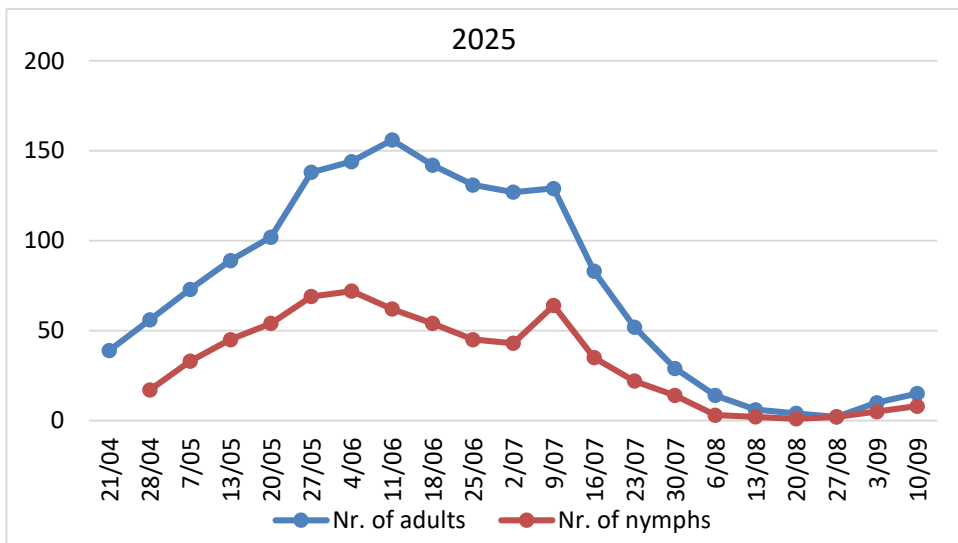
During the first year (2024), two distinct peaks in population density were recorded: the first on 14 May and the second at the end of June (Figure 1). The sharp decline observed after mid-May was associated with intense rainfall events, which likely dislodged adults and nymphs from rose shoots. Maelzer (1977) similarly reported up to 100% mortality of *M. rosae* populations during heavy precipitation. In 2025, the first aphid infestations appeared approximately ten days later than in 2024, due to low early spring temperatures (<5°C) that delayed pest emergence. However, the absence of heavy rainfall and the presence of moderate, evenly distributed precipitation created favorable conditions for sustained aphid development until mid-July (Figure 1). Comparable trends have been reported by Razdoburdin et al. (2014) and Amin et al. (2017), confirming that both temperature and precipitation significantly influence the temporal dynamics of aphid populations.

The prolonged maintenance of more moderate temperatures during the second year, combined with moderate and evenly distributed rainfall, favoured the development of the pest (Figure 2). Unlike the first year, the first attacks were recorded slightly later, in the second ten-day period of April. The reason for this is isolated days with low temperatures below 5°C at the end of March and the beginning of April. With the gradual and prolonged maintenance of average daily temperatures after mid-April, the multiplication of the green rose aphid also began. The absence of heavy rainfall and the presence of moderate temperatures created favourable conditions for aphid development; since there were no rains that could dislodge aphids from the shoots, the population remained stable from the end of May until mid-July.



**Figure 1.** Population dynamics of *Macrosiphum rosae* L. in the first year.

After that, with the increase in average daily temperatures, a gradual decline in the pest population was noted. Several researchers have indicated that climate conditions and geographic location can influence pest population dynamics (Razdoburdin et al. 2014; Amin et al. 2017). Elevated temperatures may decrease *Macrosiphum rosae* L. populations (Ahmed & Aslam, 2000). In this study, the population density of the green rose aphid was higher in 2022, when environmental conditions were more favourable. Further research will focus on analysing the factors that affect feeding behaviour and population density.



**Figure 2.** Population dynamics of *Macrosiphum rosae* L. in the second year.

### ***Efficacy of Beauveria bassiana-based treatments***

The bioinsecticidal treatments significantly affected the density of *M. rosae* populations compared with the untreated control (Table 2). A single foliar application of **Naturalis®** achieved an average efficacy of 56–57% across both years, while *B. bassiana* strain 214 reduced aphid density by 24–28%. Double applications markedly enhanced efficacy, reaching **77–83%** for Naturalis® and **44–48%** for strain 214. These results indicate that the commercial strain (ATCC 74040) exhibited superior virulence under field conditions, but the local strain 214 still demonstrated consistent moderate control, confirming its biocontrol potential.

Analysis of variance (ANOVA) showed that **product, application frequency, and days after treatment (DAT)** had highly significant effects ( $p < 0.01$ ), whereas **year** did not significantly influence efficacy ( $p > 0.05$ ). The interaction between product and application frequency was significant, indicating that the benefit of double application differed between strains. Tukey's HSD post-hoc test confirmed that *Naturalis®* (*double application*) performed significantly better than all other treatments (Table 2). The effect size ( $\eta^2$ ) analysis revealed that product type accounted for 71.9% of the total variance in efficacy, followed by application frequency (22.2%), while year and interaction effects contributed minimally (<1%).

**Table 2.** Efficacy of the tested products over the period (%).

| Application regime | Product                       | 2024 – 3 DAT | 2024 – 5 DAT | 2024 – 7 DAT | 2025 – 3 DAT | 2025 – 5 DAT | 2025 – 7 DAT |
|--------------------|-------------------------------|--------------|--------------|--------------|--------------|--------------|--------------|
| Single application | Naturalis®                    | 51           | 55           | 63           | 53           | 57           | 61           |
| Single application | <i>B. bassiana</i> strain 214 | 22           | 26           | 35           | 20           | 24           | 29           |
| Double application | Naturalis®                    | 70           | 78           | 83           | 72           | 76           | 82           |
| Double application | <i>B. bassiana</i> strain 214 | 42           | 45           | 48           | 35           | 39           | 44           |

DAT – days after treatment.

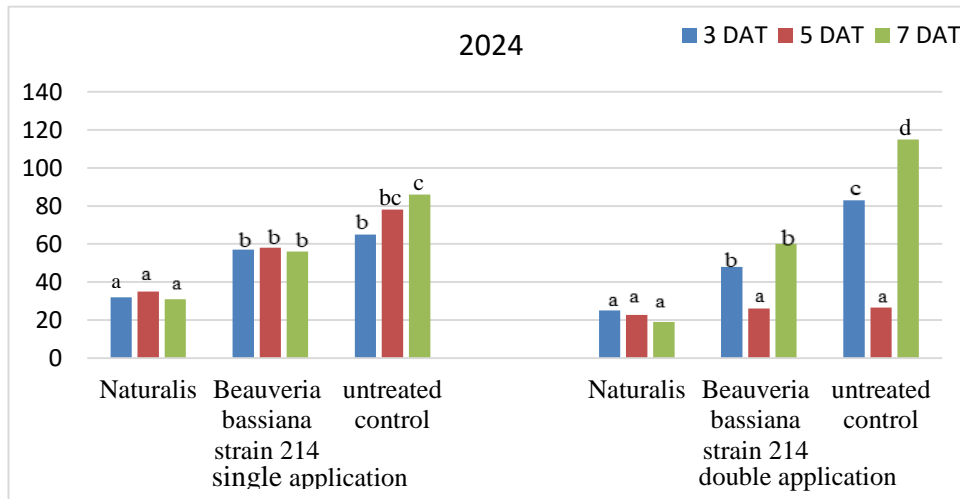
\*Efficacy is calculated based on the number of live insects in the untreated control

\*\*DAT- days after treatment

A single treatment with *Beauveria bassiana* strain 214 managed to reduce the population of the green rose aphid by up to 35% on the seventh day after treatment in 2024 (Figure 3). The efficacy of both products increased after double application. Naturalis® applied twice over 10 days achieved the best results in controlling the green rose aphid and managed to reduce the population by up to 83% in 2024. The application of *Beauveria bassiana* strain 214 was able to reduce the pest population by up to 44% in 2025 and up to 48% in 2024. Low average daily temperatures from March to mid-April delayed the development not only of rose plants but also the appearance of the green rose aphid.

The first treatment in the second year was carried out on April 25, when the pest began to appear. In the second year, the product Naturalis® maintained a leading position in efficacy, managing to reduce the population of the green rose aphid by 61% with a single application and 82% with a double treatment. In the

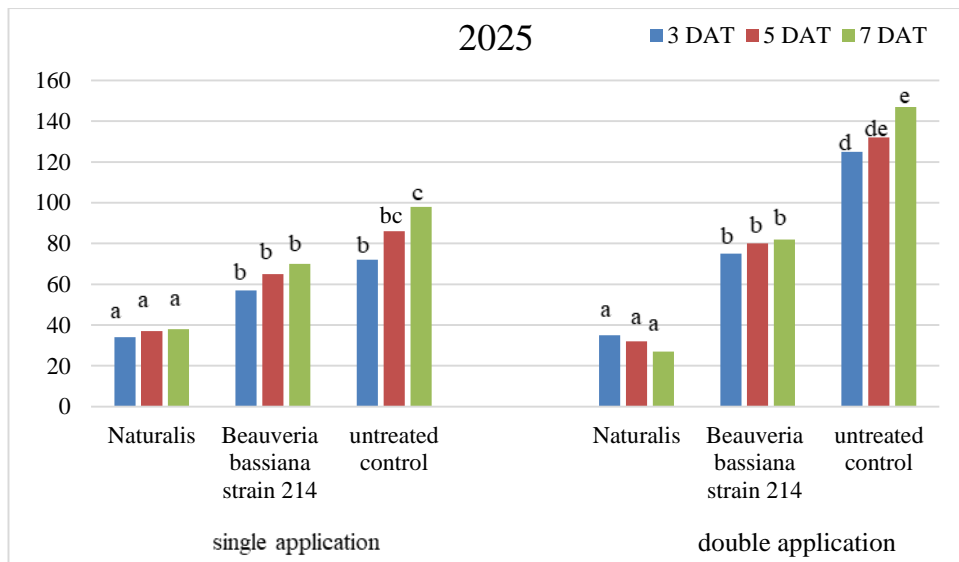
variants treated with *Beauveria bassiana* strain 214, the efficacy ranged from 29% for a single treatment to 44% for a double application (Table 2, Figure 4). The observations from the present study are consistent with the findings of other authors.



\*Values with the same letters do not differ significantly.

\*\*DAT- days after treatment

**Figure 3.** Number of live *M. rosae* individuals after treatment in the first year (2024).



\*Values with the same letters do not differ significantly.

\*\*DAT- days after treatment

**Figure 4.** Number of live *M. rosae* individuals after treatment in the second year (2025).

Filho et al. (2011) demonstrated that *Beauveria bassiana* shows an efficacy of 57-60% against insects with piercing-sucking mouthparts. Dara (2017) added that the efficacy of the biological agent *Beauveria bassiana* depends on the strain used. Eidy et al. (2016) investigated the entomopathogenic potential of *Beauveria bassiana* and *Lecanicillium lecanii* (Zimmermann) Zare & W. Gams (*Verticillium lecanii*) against the green rose aphid under laboratory conditions and found that *L. lecanii* has higher virulence compared to *Beauveria bassiana*. The lethal doses causing 50% mortality in adult *M. rosae* were  $1.38 \times 10^4$  spores/ml for *Verticillium lecanii* and  $2.66 \times 10^5$  spores/ml for *Beauveria bassiana*. For *V. lecanii*, the lethal effect was observed between the 1st and 3rd day after treatment, while for *Beauveria bassiana* it occurred after the second day of treatment. The authors also reported that as the concentration of the solution increased, the time for the lethal effect to manifest shortened. Loureiro and Moino (2006) observed that *B. bassiana* causes 100% mortality of *M. persicae* at a concentration of 106 spores/ml on the seventh day after treatment. Abd El-Salam and El-Hawary (2011) reported 100% mortality of adult *A. carvicora* when treated with *V. lecanii* at a concentration of 106 spores/ml on the third day after application. Data from Saranya et al. (2010) indicated that *V. lecanii* achieves 100% mortality at a concentration of  $10^7$  spores/ml, while *B. bassiana* caused 96% mortality at a concentration of 108 spores/ml on the seventh day after treatment.

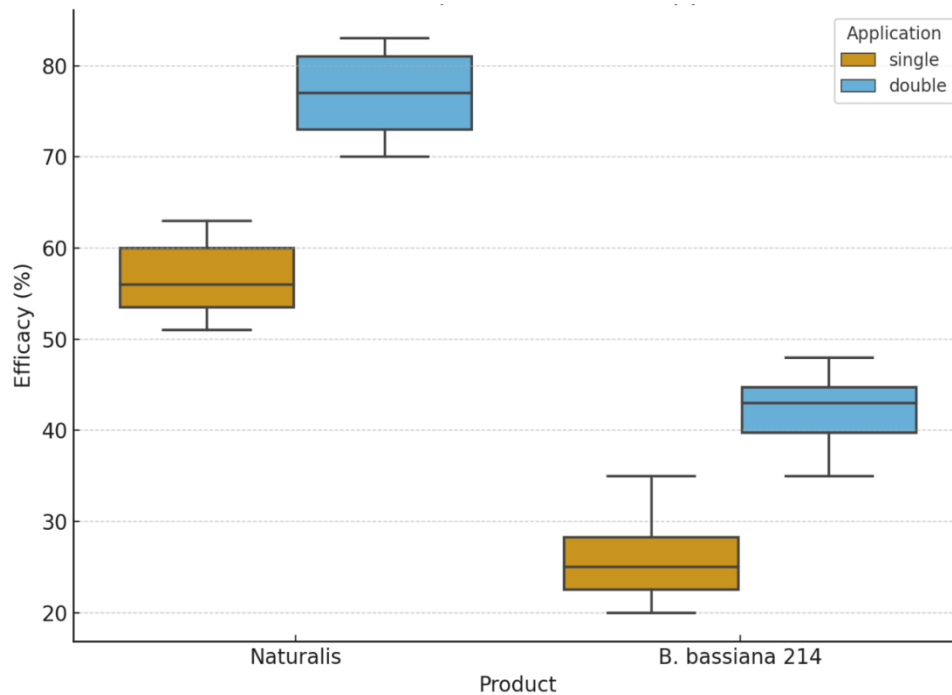
#### ***Efficacy of Beauveria bassiana 214 and the Commercial Product Naturalis under Different Application Regimes***

The efficacy of the two entomopathogenic fungal products, *Naturalis* and *Beauveria bassiana* 214, varied significantly depending on both the product type and the number of applications (Figure 5). The boxplot clearly demonstrates that *Naturalis* achieved substantially higher efficacy values than *B. bassiana* 214 across both application regimes.

Under a **single application**, *Naturalis* recorded a median efficacy of approximately **56%**, with relatively narrow variability (range 51–63%), whereas *B. bassiana* 214 exhibited a markedly lower efficacy, averaging around **25%** (range 20–35%). Following a **double application**, efficacy increased for both products, though to different extents: *Naturalis* reached a median of **77%** (range 70–83%), while *B. bassiana* 214 improved modestly to **43%** (range 35–48%).

These findings demonstrate that double applications enhance the insecticidal efficacy of both fungal products, though the improvement is markedly greater for *Naturalis*. The superior performance of *Naturalis* may be attributed to the optimized formulation and the higher pathogenic potential of the commercial *B. bassiana* ATCC strain, while the lower efficacy of the native isolate 214 may reflect differences in conidial germination rate, UV tolerance, or environmental adaptability.

Boxplots display the median, interquartile range, and minimum–maximum values. The data demonstrate that both products achieved higher efficacy following double application, with *Naturalis*® consistently outperforming *B. bassiana* 214.



**Figure 5.** Efficacy (%) of the commercial product *Naturalis*® and the native isolate *Beauveria bassiana* 214 under single and double application regimes.

**ANOVA Analysis**

The two-way ANOVA indicated that both **product type** and **application regime** had a highly significant effect on efficacy ( $p < 0.001$  for both factors) (Table 3). The commercial product *Naturalis* consistently exhibited higher efficacy compared to the native *Beauveria bassiana* 214 isolate. Similarly, the efficacy of both products significantly increased following a double application relative to a single treatment, confirming the cumulative benefit of repeated application.

**Table 3.** Two-way ANOVA results for treatment efficacy of the commercial biocontrol product *Naturalis* and the native isolate *Beauveria bassiana* 214 under single and double applications.

| Factor                              | Interpretation   | Expected Significance           |
|-------------------------------------|--|---------------------------------|
| Product                             | Naturalis consistently higher efficacy than <i>B. bassiana</i> 214   | $p < 0.001$                     |
| Application                         | Double > Single for both products  | $p < 0.001$                     |
| Interaction (Product × Application) | Effect of double application is stronger in <i>Naturalis</i> than in <i>B. bassiana</i> 214 (difference in slopes) | $p < 0.05$ (likely significant) |

A significant **interaction** between product and application ( $p < 0.05$ ) was also observed, indicating that the effect of double application was more pronounced for *Naturalis* than for *B. bassiana* 214. This interaction suggests that formulation quality or strain-specific characteristics influence the degree of improvement achieved through repeated treatments. Overall, these results demonstrate that while both the product type and the number of applications significantly affect biocontrol efficacy, *Naturalis* exhibits superior performance and a stronger response to repeated application compared to the native isolate.

#### ***Climatic influence on efficacy***

The lower aphid density and reduced efficacy observed in 2024 corresponded with excessive rainfall during the application period, which likely limited spore persistence and contact with insect hosts. In contrast, the more stable weather in 2025 favored prolonged fungal activity. These results confirm that environmental factors—particularly relative humidity and temperature—play a key role in determining the success of entomopathogenic fungi in open-field conditions (Lacey et al., 2001; McKinnon et al., 2017). Therefore, the timing of applications should consider both pest phenology and microclimatic conditions to maximize fungal infection and sporulation.

### **CONCLUSION**

The population dynamics of the green rose aphid were influenced by the climatic conditions prevailing during the study period. The native *Beauveria bassiana* strain 214 demonstrated promising efficacy against the pest, confirming its potential as a biological control agent. However, the effectiveness of the entomopathogenic fungus was strongly dependent on the intensity and frequency of application. The commercial formulation *Naturalis*® exhibited superior and more consistent efficacy compared to the native *B. bassiana* 214 strain, suggesting that formulation optimization and repeated applications could further enhance the performance of local isolates under field conditions.

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