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# Optimizing calendula yield, quality, and *Empoasca decipiens* pest control with NPK, PGPR, and eco-friendly pesticides

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## Abstract

*Calendula officinalis*, noted for its therapeutic and aesthetic qualities, requires sustainable cultivation to meet rising demand. The research was conducted at the Experimental Farm of Qaha Agriculture Station, Qalyoubia Governorate, under the Plant Protection Research Institute of the Agriculture Research Center, Egypt, during the 2022-2023 and 2023-2024 seasons. The study sought to improve the quality and yield of calendula plants while controlling pest populations, particularly *Empoasca disciplines*, a significant economic pest that inflicts considerable damage on horticultural crops. This was achieved through two primary factors: the first involving three fertilizer treatments (recommended NPK dose as control, PGPR, and NPK combined with PGPR), and the second encompassing alternative pesticides (administered with and without alternative pesticides). The alternative pesticides consist of six environmentally benign options: Giskanim 1% EC, Sulfur 30% Liquid, Brave A M 6% SL, KZ Oil 95% EC, Potassium Agricultural Soap, and Top Perfect 82% EC. The utilization of all alternative pesticides and NPK with PGPR markedly improved growth performance, flowering characteristics, and chemical composition (total chlorophyll in leaves, total carotenoids, total flavonoids in dry ray flowers, percentages of nitrogen, phosphorus, and potassium, and total carbohydrates in leaves) while diminishing pest populations of *Empoasca discipiens* compared to the absence of alternative pesticides and other treatments.

**Keywords:** *Calendula officinalis*, Pot marigold, alternative pesticides, carotenoids, flowering quality,

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## 1. Introduction

*Calendula*, an annual plant recognized for its medicinal and ornamental value, is commonly referred to as the English marigold, the bride of the sun, pot marigold, butterwort, and bull flower, and is classified within the Asteraceae family as a commercially viable floral species. The striking yellow-to-orange pigmentation of the flowers renders them particularly desirable for utilization as cut flowers. Marigold, which holds popularity both as a decorative flower and as a medicinal entity, encompasses a spectrum of phytochemicals and essential oils such as linalyl acetate, limonene, and linalool, thereby establishing its appropriateness for diverse ornamental applications and horticultural settings [1,2]. Moreover, calendula's phytochemical constituents are associated with various biological activities, including immuno-stimulant, wound healing, hepatoprotective, and anti-amylase effects, contributing to its esteemed status as a medicinal plant [3–5]. On the other hand, the yellow petals of calendula are recognized as a potent therapeutic agent for treating inflamed and erythematous skin, endowed with antiseptic and medicinal attributes that inhibit the proliferation of infections and expedite the healing process [6,7]. Calendula cream has demonstrated efficacy in addressing conditions such as acne and diaper rash, functioning as a bactericide and antiseptic, and possessing anti-inflammatory, antigenotoxic, chemoprotective, antitumor, antibacterial, and antiseptic characteristics [8,9]. The flavonoids found within *C. officinalis* demonstrate considerable antioxidant properties. Concurrently, the petals in it and pollen grains of calendula are abundant in triterpenoid esters, carotenoids, flavaxanthin, auroxanthin, and various other compounds that are utilized in industrial coatings and the synthesis of nylon [10–13].

Furthermore, investigators are examining plant growth-promoting rhizobacteria (PGPR), which establish themselves on plant roots and enhance the potential for plant development. PGPR is integral in sustaining soil fertility, diminishing reliance on agricultural chemicals, improving plant growth, lowering the necessity for synthetic fertilizers, and fostering sustainable farming practices. They also exhibit ACC deaminase activity, alleviating ethylene levels induced by salinity stress [14–16].

*Empoasca decipiens Paoli* represents a deleterious insect pest that adversely impacts ornamental and vegetable crops globally. This particular species contributes to the dissemination of phytopathogenic viruses and can elicit phytotoxic repercussions, adversely affecting agricultural productivity [17]. A comprehensive examination of the life cycle characteristics of the green leafhopper is undertaken to develop effective management strategies. The utilization of life tables serves to facilitate the comparison and prediction of pest population dynamics under varying environmental conditions [18,19].

Integrated pest management (IPM) and sustainable agricultural methods are recognized as environmentally friendly alternatives to chemical pesticides. These methods focus on controlling *Empoasca discipiens* populations in calendula while reducing ecological harm. Applying these methods significantly alters the *Empoasca discipiens* population dynamics in calendula cultivation. The primary objective is to decrease pest populations and lessen environmental impact [20]. Chemical Control: Selective pesticides like Giskanim and Top Perfect effectively target *E. discipiens* without harming beneficial insects. Biological Control: The employment of indigenous predatory species and entomopathogenic fungi significantly augments pest management efficacy, as evidenced in various crops. Cultural Practices: Approaches such as crop rotation and habitat manipulation effectively interrupt the life cycle of *E. discipiens*, resulting in diminished infestation levels. Economic Viability: The adoption of Integrated Pest Management (IPM) strategies correlates with enhanced agricultural yields and superior economic performance relative to traditional methodologies [21,22].

The evaluation of sulfur 30% liquid, Brave A M 6% SL, KZ Oil 95% EC, potassium agricultural soap, and Top Perfect 82% EC indicates significant variances in both environmental impact and pest control effectiveness. Sulfur is noted for its minimal environmental toxicity and successfully manages pests such as the alfalfa weevil and pistachio psylla, achieving efficacy rates of up to 87.2% with multiple applications [23]. Conversely, while effective, Brave A M and KZ Oil may present greater environmental hazards due to their chemical formulations. Potassium agricultural soap and Top Perfect are recognized as more eco-friendly options and demonstrate encouraging results against various pests. However, the efficacy data for these alternatives are not as comprehensive as that for sulfur. Sulfur 30% Liquid serves as a potent fungicide and pesticide, effectively regulating aphid infestations while minimizing phytotoxic effects in pome fruit orchards [24,25]. It enhances phytobiome vitality, augments agronomic productivity, and mitigates detrimental impacts on beneficial organisms, thereby establishing itself as a sustainable approach to nutrient management [26–30].

Moreover, Brave A M 6% SL and KZ Oil are effective pest control products, but their chemical formulations raise environmental concerns. KZ Oil 95% EC is effective during dormant periods but has a higher toxicity level [24,31] Potassium Agricultural Soap targets soft-bodied insects and is environmentally friendly. Top Perfect 82% EC combines active components, improving pest control efficacy, but potentially increasing ecological concerns. Sulfur is also effective, but a balance between efficacy and ecological footprint is crucial. These environmentally sustainable alternatives can help manage aphids and promote sustainable agricultural practices. Despite higher production costs, organic biopesticides are preferred due to long-term risks to human health and the environment [32,33].

These ecologically friendly methods help manage aphids and promote sustainable agriculture practices. Reduced insect damage allows chrysanthemums to thrive, increasing floral yield and quality [34]. Incorporating these products into Integrated Pest Management (IPM) systems allows for a more comprehensive pest control strategy, reducing dependency on dangerous chemical pesticides. Pesticides offer long-term dangers to human health and the environment; hence, regulatory bodies prefer organic biopesticides, despite greater production costs [34]. This research was conducted to investigate the impact of fertilizer treatments and a program of alternative pesticides—either separately or in combination—on the growth, flowering, and chemical composition of Calendula plants. In addition, to control the population of the leaf hopper *Empoasca discipiens* Paoli (nymphs and adults) (Order: Homoptera, Family: Cicadellidae).

## 2. Materials and methods

### 2.1. Experimental site

A field experiment was located at the Experimental Farm of Qaha Agriculture Station, Qalyoubia Governorate, Plant Protection Research Institute, Agriculture Research Center, Ministry of Agriculture, during the winter seasons of 2022-2023 and 2023-2024. The averages of weather parameters over the study site during the experimental period are given in Figure (1). Data sourced from the Central Laboratory for Agricultural Climate (CLAC), Agricultural Research Center (ARC), Egypt.

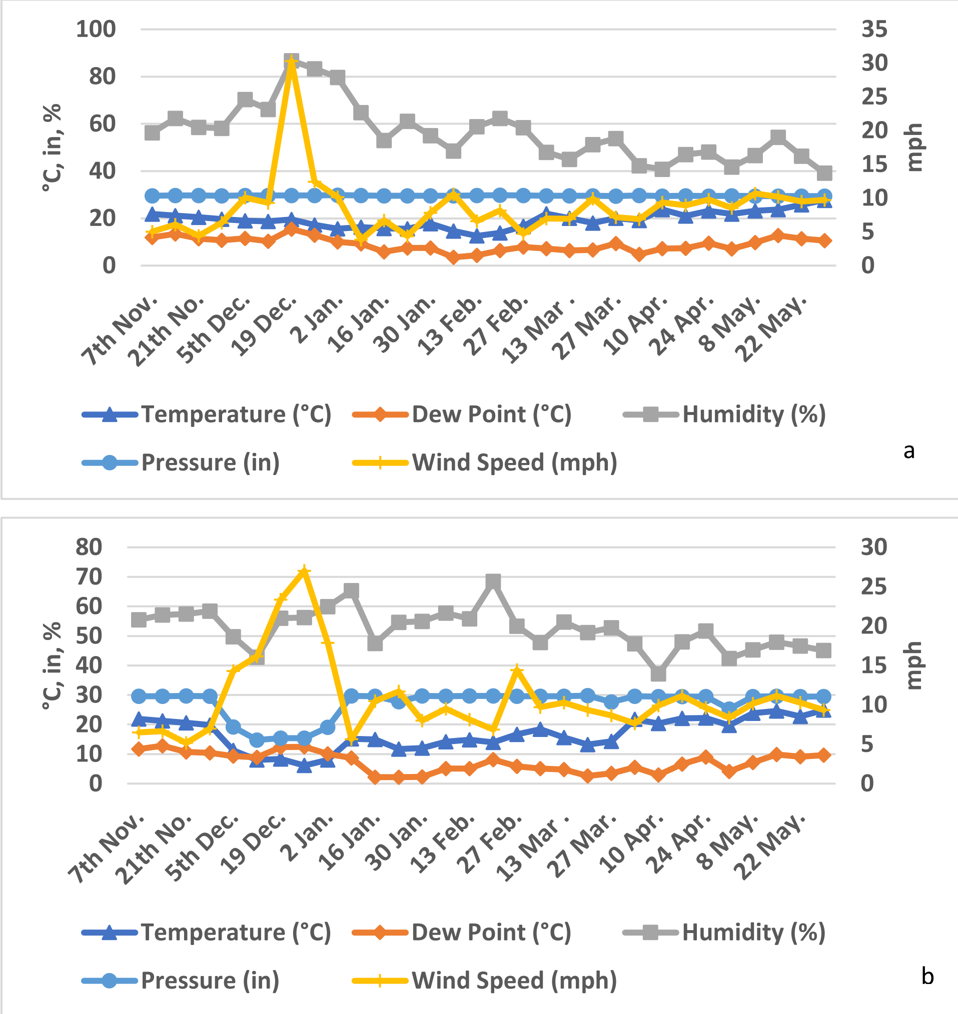



**Figure 1. Climatic variables for Qaha Agriculture Station, Qalyoubia Governorate, Egypt, for the (a) 2022/2023 and (b) 2023/2024 seasons.**

Before planting, the experimental soil was analyzed physically and chemically according to [35,36]. The physicochemical analyses of the soil are shown in Table 1.

**Table 1.** Physicochemical analyses of the experimental soil before planting during the two winter seasons of 2022–23 and 2023–24.

| Properties | Values | | Properties | Values | |
|---|---|---|---|---|---|
| | 2022 | 2023 | | 2023 | 2024 |
| Sand (%) | 22.44 | 21.26 | Organic matter % | 1.37 | 1.46 |
| Silt (%) | 29.10 | 29.41 | Available N (ppm) | 67.65 | 70.98 |
| Clay (%) | 48.46 | 49.33 | Available P (ppm) | 5.27 | 5.79 |
| Textural class | Clay | Clay | Exchangeable K (ppm) | 59.53 | 58.64 |
| pH | 7.38 | 2.49 | Wilting point (%) | 46.09 | 45.82 |
| EC ($dSm^{-1}$) (1:10) | 2.2 | 2.2 | Available water (%) | 24.41 | 21.55 |
| CaCO3 % | 2.62 | 2.49 | Bulk density (g $cm^{-3}$) | 1.22 | 1.21 |
| Soluble anions (soil paste, mmolc $L^{-1}$): | | | Soluble cations (soil paste, mmolc $L^{-1}$): | | |
| $CO_3^{-2}$ | 0.00 | 0.00 | $Ca^{+2}$ | 5.20 | 5.40 |
| $HCO_3^-$ | 5.40 | 5.90 | $Mg^{+2}$ | 3.00 | 4.30 |
| $Cl^-$ | 7.60 | 8.60 | $Na^+$ | 6.40 | 7.20 |
| $SO_4^{-2}$ | 6.90 | 7.50 | $K^+$ | 5.30 | 5.10 |

### 2.2. Experimental design

The experimental protocol was based on a factorial design using a completely randomized design with four replicates. Each plot consisted of 5 rows, each 3.5 m long and 0.7 m wide. The combination of treatments consisted of two main variables, namely fertilizer applications and an alternative pesticide program. The total experimental area was 1200 $m^2$, with each treatment occupying 400 $m^2$.

### 2.3. Fertilizer treatments

Three fertilizer treatments were applied: (I) 100% NPK of the recommended dose, (II) 100% PGPR, and (III) 50% NPK of the recommended dose + 50% PGPR. The mineral source of NPK was ammonium sulfate "20.6% N" at 300 kg/ha, calcium superphosphate "15.5% $P_2O_5$" at 238 kg/ha, and potassium sulfate "48% $K_2O$" at 100 kg/ha. N and K fertilizers were applied thrice post-transplantation at 15-day intervals, while P was applied once during soil preparation.

PGPR (nitrogen-fixing microorganisms (*Azospirillum brasilense*), phosphate-solubilizing microorganisms (*Bacillus megaterium* var. phosphaticum), and potassium-solubilizing microorganisms (B. *circulans*) sourced from the Soil Microbiology Laboratory at the Agricultural Research Center in Egypt, according to the [37] method.

### 2.4. Alternative pesticide program treatments

The alternative pesticides program variable was designed to control *Empoasca discipiens* Paoli and was assigned into two treatments: (I) No application of the alternative pesticides program as a control, and (II) with application of the alternative pesticides program. The trade name, active ingredient, and application rate of the alternative pesticides are shown in Table (2).

**Table 2. The alternative pesticides**

| Trade name | Active ingredient | Rate of application ml /100-liter water (Recommended) |
|---|---|---|
| Giskanim 1% EC | Azadirachtin | 125 |
| Sulfur 30% Liquid | 24% Sulfur + 6% Calcium | 400 |
| Brave A M 6 % SL | Orange oil (d-limonene) | 400 |
| KZ oil 95% EC | Mineral oil | 1000 |
| Potassium agricultural soap | Potassium salts | 1000 |
| Top Perfect 82% EC | Jojoba oil | 250 |

The listed alternative pesticides were periodically sprayed every 10 days in rotation, starting from the 25$^{th}$ of October until the end of each season (5$^{th}$ May). The average of inspection data in the table for three days was inspection data after (2,7,10) days and consists of 10 plants/ replicate = 40 plants/ treatment and control, using the direct counting technique for inspection. Standard precautions were taken during vegetation, such as protecting against pests and turning the dirt upside down, to ensure all agricultural practices required to create seedlings were met.

### 2.5. Experimental protocol

Pot marigold plant (*Calendula officinalis* L.) seeds were obtained by the Orman Garden Giza, Egypt, and sown in the mid of October in both seasons in a seedbed mixture of vermiculite and peat moss (2:1). Uniform calendula seedlings, 45 days old and 15 cm length were transplanted under field conditions at a spacing of 25 cm from each other. Except for fertilization and pesticides, all agricultural practices for calendula growing were implemented, according to the Egyptian Ministry of Agriculture and Land Reclamation.

### 2.6. Recorded data

#### 2.6.1. Morphological and floral measurements

At the end of each growing season, morphological parameters including plant height (cm), number of main and lateral branches/plant, root length (cm), and fresh weight (g) per plant were measured. During the flowering stage, some flowering parameters were stated, such as flowering start (days until the first flower opens), flowering period (days from the first flared flowering head until the end of the flowering), and number of inflorescences per plant (7 cuts), inflorescences diameter (cm) per plant, fresh and dry inflorescences weight (g) per plant.

### 2.6.2. Biochemical measurements

Total chlorophyll in leaves was determined according to the method described by Moran [38]. Total carotenoids in fresh petals (mg/g f.w.) were determined spectrophotometrically in the acetone extract according to the methods of Dere et al. [39]. Moreover, the total flavonoids (mg/g d.w.) were measured in dried flower heads according to Bacot [40]. Total carbohydrates (%) were analyzed for total carbohydrates (%) using the method of Dubois et al. [41]. In addition, the total content of nitrogen, phosphorus, and potassium % in the leaves was examined according to the Cotteine *et al.* [42] method.

### 2.6.3. Measurements of the *Empoasca discipiens* pest population

The leafhopper, *Empoasca discipiens*, infestations and phytoplasma were monitored on calendula plants throughout both growing seasons. Ten plants were randomly selected from each treatment plot three times a month. This monitoring began a month after sowing in the seed bed in mid-October and concluded a week after irrigation stopped in May. To assess the *Empoasca discipiens* population density, three leaves or inflorescences were randomly chosen from each selected plant, representing the top, middle, and lower levels. The number of *Empoasca discipiens* nymphs and adults on each leaf was directly counted using a magnifying hand lens with 10x magnification. The percentage of *Empoasca discipiens* population reduction was calculated using the Hinderson and Tilton formula [43] as follows:

$$Reduction\ (\%) = \frac{N\ in\ control\ before\ treatment\ X\ N\ in\ after\ treatment}{N\ in\ control\ after\ treatment\ X\ N\ in\ before\ treatment}\ X\ 100 \ldots\ldots\ldots\ldots\ldots (1)$$

In addition, the number of phytoplasma was counted according to the following formula:

$$Phytoplasma\ (\%) = \frac{N\ of\ plants\ infected\ with\ phytoplasma}{Total\ number\ of\ plants}\ x\ 100 \ldots\ldots\ldots\ldots (2)$$

Where: n = Insect population, T = treated, Co = control

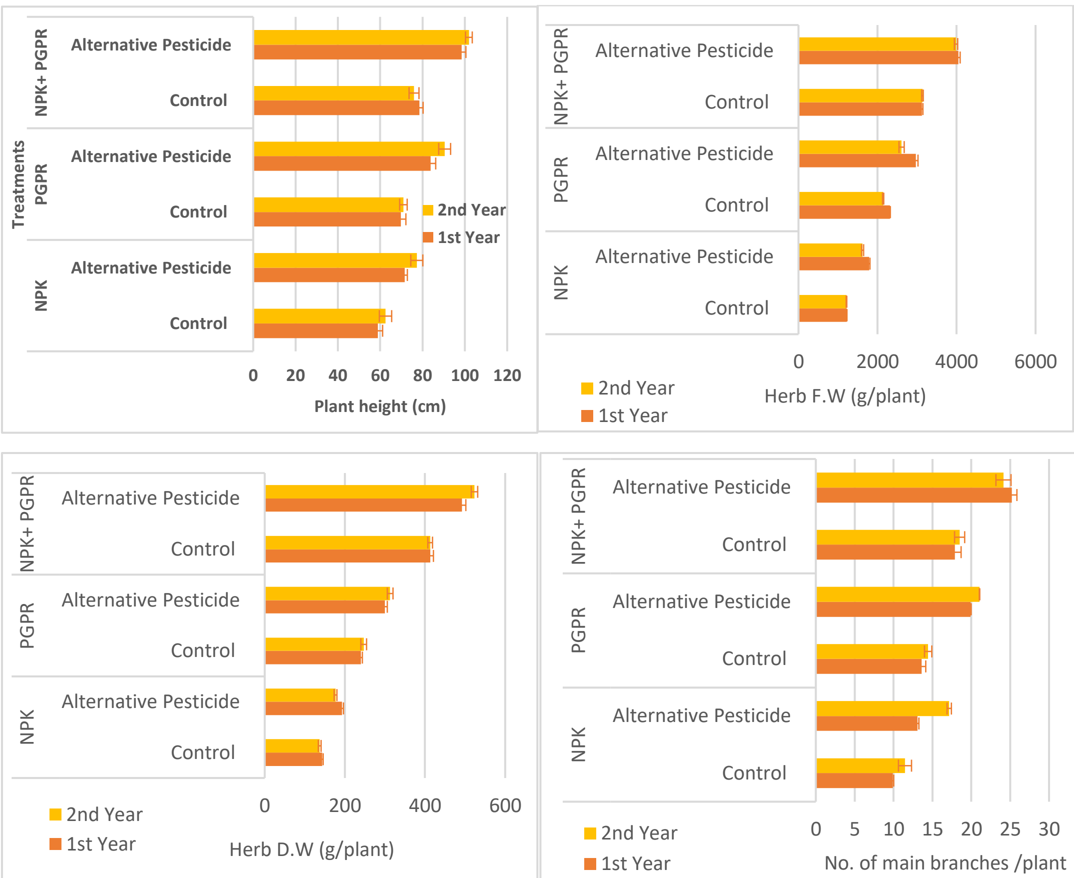
### 2.7. Statistical analysis

The COSTAT computer program was utilized for statistical analysis, and a two-factor analysis of variance according to Gomez and Gomez [44], $p \leq 0.05$ was used to determine significant differences between treatments. Data were expressed as mean ± S.D.

## 3. Results

### 3.1. Morphological measurements

The studied plant's morphological characters detected significant effects concerning the foliar spray with alternative pesticide treatments, with NPK elements and PGPR applications (Figure, 2). Compared to the control plants (NPK application and without pesticides), all treatments caused a substantial boost in all plant growth characteristics. The maximum values for both seasons of plant height were about 40.29 and 38.63%; herbs fresh weight/plant (69.79 and 69.55%), herbs dry weight/plant (70.92 and 73.78%), the number of main branches/plant (60.67 and 52.48%), the number of lateral branches/plant (75.79 and 73.01%), the roots fresh weight/plant (61.01 and 56.62%), and the root length/plant (63.98 and 67.6%) were obtained from a plant treated with NPK + PGPRs under the application of the alternative pesticides program.



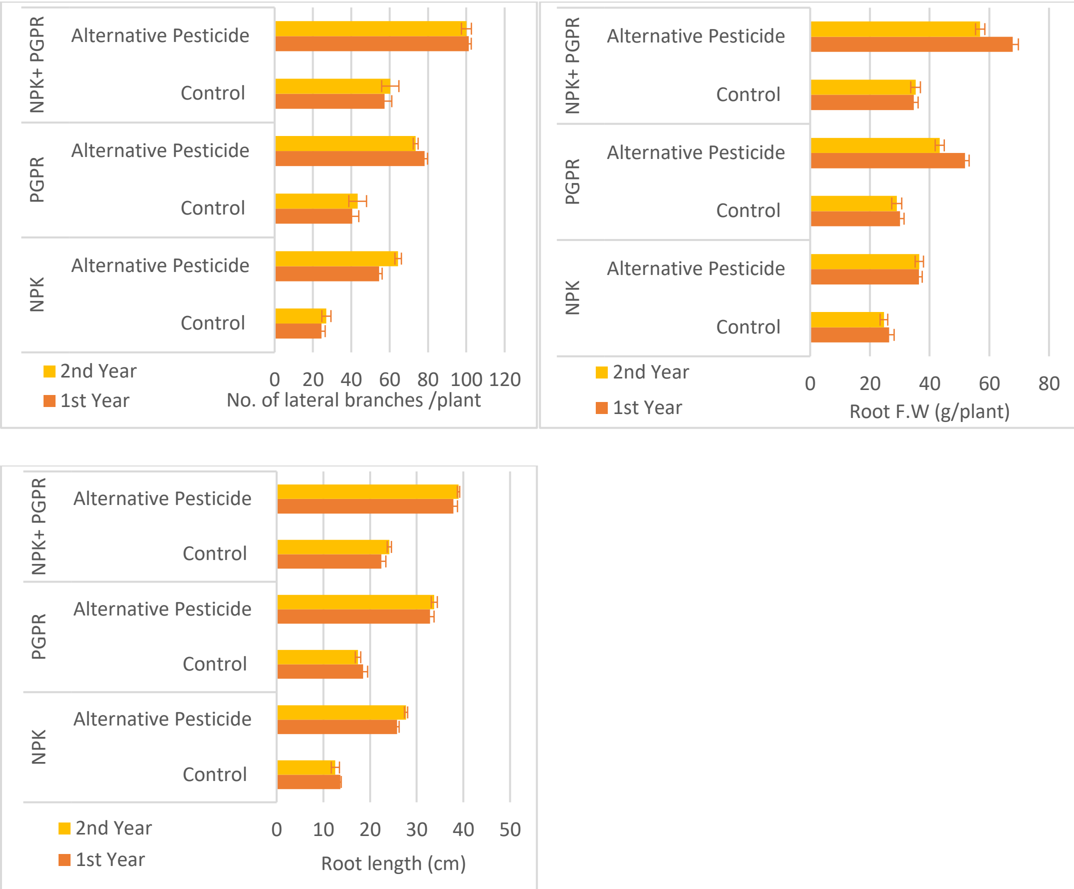



**Figure 2. Morphological characters of calendula plants. Data are presented as means ± SD.**

### 3.2. Floral measurements

Results indicated that applying the alternative pesticide treatments with NPK and PGPR yielded remarkable results in flowering performances, as shown in Figure 3. Generally, mineral fertilizer alongside biofertilizer treatments led to earlier calendula flowering than sole mineral fertilizer or biofertilizer. Under the application of the alternative pesticides program, a significant equivalence was observed between the flowering start of all plants treated with mineral NPK, PGPR, and NPK plus PGPR fertilization; the earliest time of the first flower opened occurred with NPK plus PGPR treatment, which recorded 39.7 and 39.11 days in the first and second seasons, respectively. This was significantly earlier than the control plants (NPK application and without pesticides) by about two weeks. Furthermore, in both seasons, the flowering period of the fertilized plants increased significantly compared to the control, 27.49% and 27.23%, respectively.

In addition, NPK +PGPR fertilization, alongside the pesticides program tended to achieve a higher number of inflorescences per plant by 58.37 and 53.52%, inflorescences diameter/plant by 26.75 and 28.11%, inflorescences fresh weight/plant by 58.91 and 66.66%, and inflorescences dry weight/plant by 71.54 and 70.36% compared to the control plants (NPK application and without pesticides) for both season, respectively.

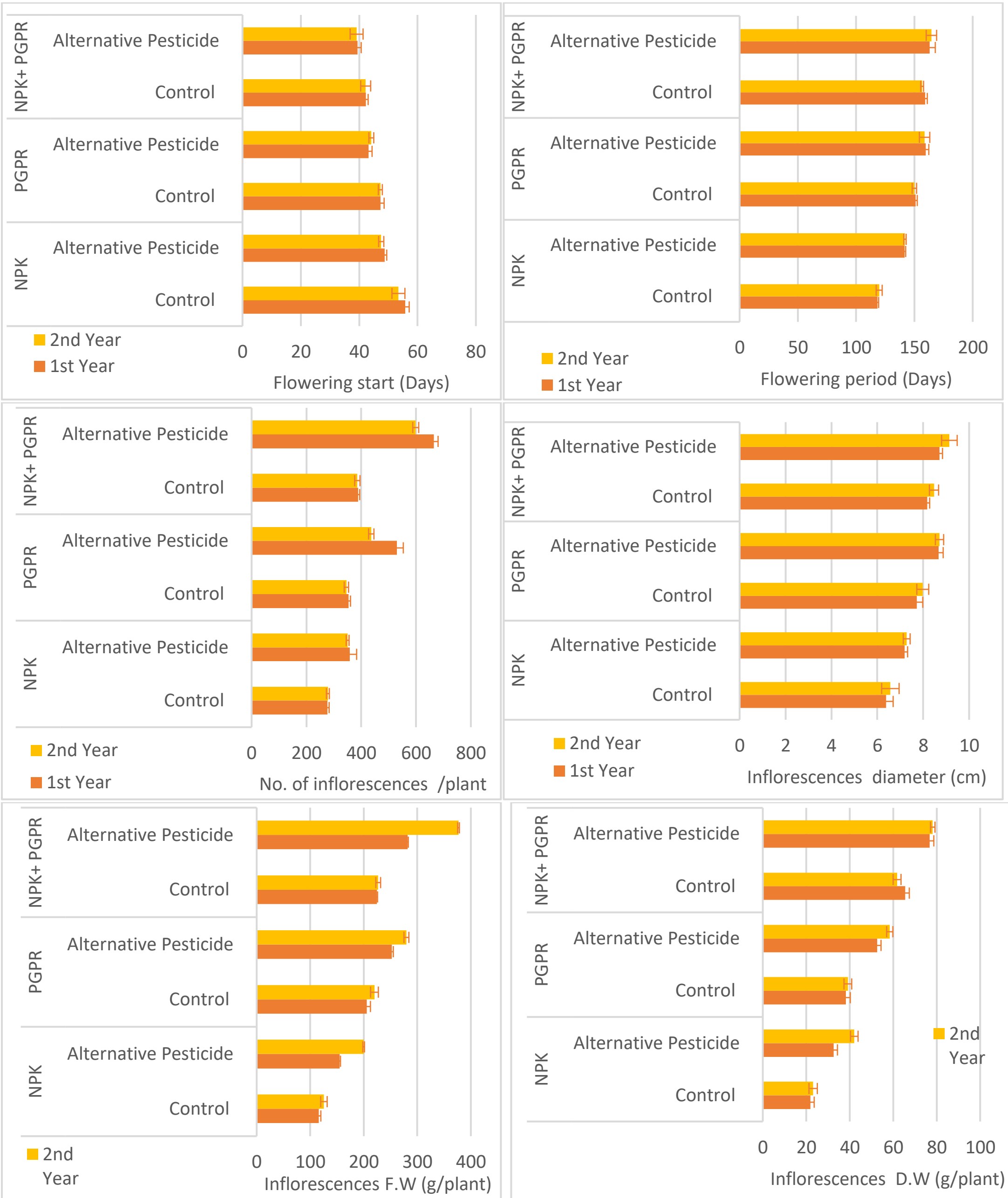



**Figure 3. Floral measurements of calendula plants. Data are presented as means ± SD.**

### 3.3. Biochemical measurements

In general, biochemical measurements include the total content of chlorophyll and carotenoids, total flavonoids, and carbohydrate, which increased with the addition of NPK mineral fertilizers along with PGPR under the application of the alternative pesticides program (Figure, 4). In both seasons, compared to the control, the total chlorophyll content was higher by 66 and 63.50%, respectively. Similarly, in both seasons, the total carotenoid content increased significantly by 29.17 and 28.67%, respectively, along with total flavonoids (57.00 and 51.15%, respectively), and total carbohydrates (70.46 and 64.61%, respectively). In addition, it was found that in both seasons, applying NPK and PGPR fertilizers to calendula plants under the alternative pesticides program resulted in higher accumulation of N, P, and K compared to the control and other treatments (Figure, 4). Compared to the control (mineral fertilizer without pesticides), the treatment with NPK mineral fertilizers plus PGPR under the alternative pesticides program yielded the highest significant increases in total N content by 58.17 and 59.20%, respectively. Similarly, P% and K% increased by 41.75 and 59.71%, respectively, in the first season. The second season showed a similar trend.

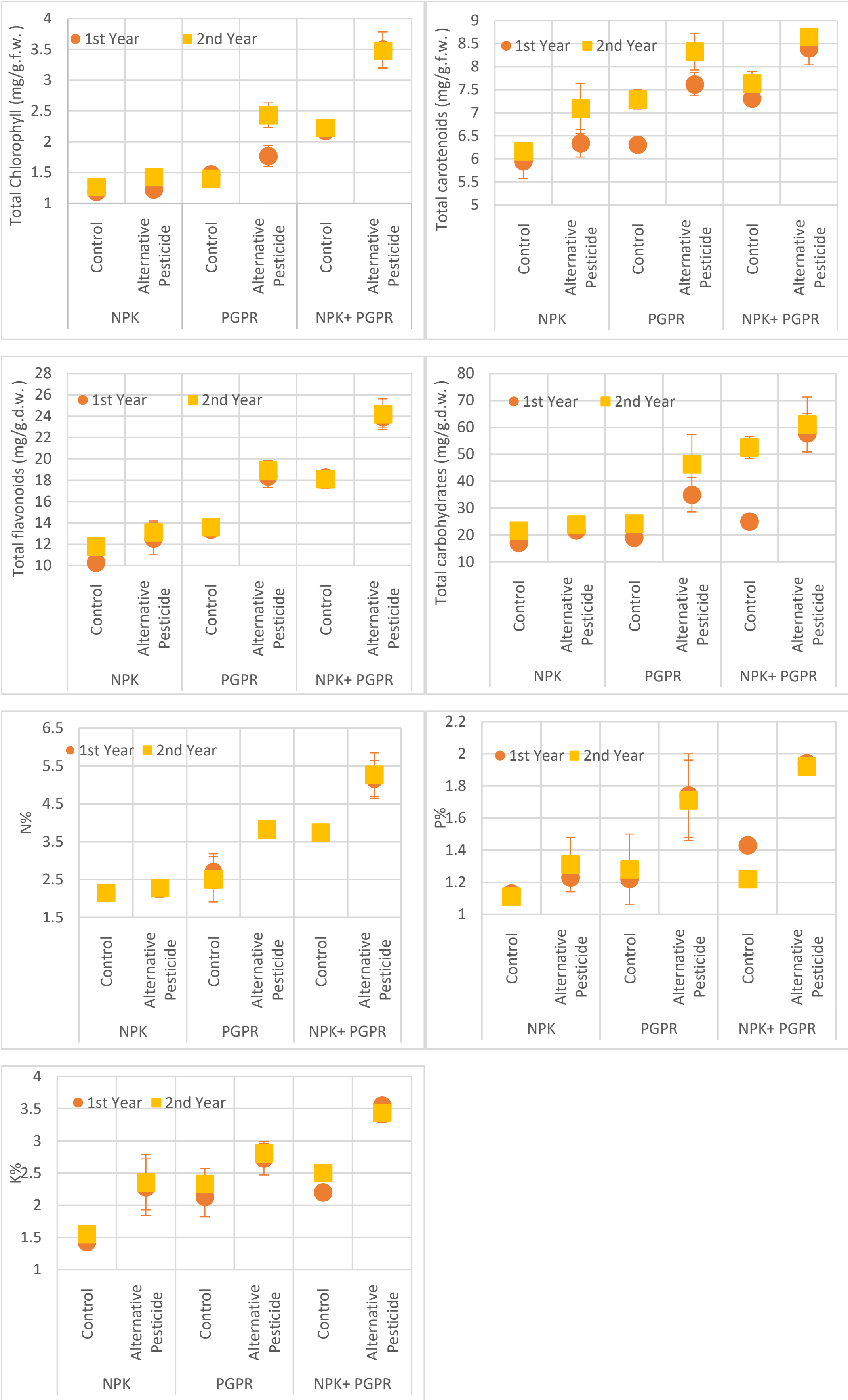



**Figure 4. Biochemical measurements of calendula plants. Data are presented as means ± SD.**

### 3.4. *Empoasca discipiens* Pest population

The population number of E. *discipiens* found on calendula plants during the two consecutive growing seasons of 2022–2023 and 2023–2024 is examined in detail in Table 3. The information is arranged according to the date of the inspection, usually at 10-day intervals between October and May, and classified according to three distinct treatment applications: NPK, PGPR, and NPK+PGPR.

Data in Table (3) showed that the population range of *E. discipiens* under the application of NPK was 14 and 39 on Oct.25$^{th}$ and Feb. 15$^{th}$ in 2022/2023, respectively. In contrast, the population ranged from 8 to 35 on Oct.25$^{th}$ and Jan. 5$^{th}$ in 2023/2024, respectively. The highest population of *E. discipiens* treatment NPK recorded 38 and 39, as well as 35 and 34 on Jan. 5$^{th}$ and Feb. 15$^{th}$ during the 2022/2023- 2023/2024 seasons, respectively. Moreover, the population of *E. discipiens on* calendula under the application of PGPR ranged between 8 and 36 on Oct.25$^{th}$ and Jan. 5$^{th}$ in the 2022/2023 season, respectively. Also, the population ranged from 6 to 32 on Oct.25$^{th}$ and Mar. 15$^{th}$ in the 2023/2024 season, respectively. The highest population of *E. discipiens* treatment PGPR was 36 & 30 and 30 & 32 on Jan. 5$^{th}$ and Mar. 15$^{th}$ during the 2022/2023 and 2023/2024 seasons, respectively. On the other hand, under the application of NPK plus PGPR, the population of *E. discipiens* ranged between 6 and 33 on Oct.25$^{th}$ and Jan. 25$^{th}$ in 2022/2023, respectively. However, the population ranged from 3 to 32 on Oct.25$^{th}$ and Mar. 25$^{th}$ in 2023/2024, respectively. The highest population of *E. discipiens* treatment NPK + PGPR was 33 and 25 on Jan. 25$^{th}$ and Mar.25$^{th}$ during the 2022/2023 season, respectively, where the highest population was 24 and 32 at Feb. 15$^{th}$ and Mar. 25$^{th}$ during the 2023/2024 season, respectively.

**Table 3. Population fluctuations of *Empoasca decipiens* Paoli on Calendula plant without application of alternative pesticides.**

| Date of Inspection | Total number of *E. discipiens* | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1st season (2022/2023) | | | | 2nd season (2023/2024) | | | |
| | NPK | PGPR | NPK+ PGPR | Mean | NPK | PGPR | NPK+ PGPR | Mean |
| Oct. 25th | 13.84 | 7.62 | 6.31 | 9.26 | 10.84 | 5.62 | 3.33 | 6.6 |
| Nov. 5th | 26.42 | 20.2 | 9.72 | 18.78 | 23.42 | 18.2 | 7.51 | 16.38 |
| 15th | 30.32 | 25.1 | 7.01 | 20.81 | 28.32 | 23.1 | 9.78 | 20.4 |
| 25th | 15.65 | 12.1 | 15.6 | 14.45 | 15.32 | 13.1 | 13.08 | 13.83 |
| Dec. 5th | 21.16 | 18.16 | 21.04 | 20.12 | 18.16 | 19.16 | 15.87 | 17.73 |
| 15th | 21.38 | 25.38 | 14.96 | 20.57 | 18.38 | 22.38 | 16.09 | 18.95 |
| 25th | 33.6 | 31.38 | 16.34 | 27.11 | 25.38 | 29.38 | 16.31 | 23.69 |
| Jan. 5th | 37.75 | 35.53 | 16.12 | 29.8 | 34.75 | 33.53 | 18.09 | 28.79 |
| 15th | 23.16 | 23.16 | 18.87 | 21.73 | 17.16 | 24.16 | 20.65 | 20.66 |
| 25th | 20.95 | 20.95 | 20.15 | 20.68 | 23.95 | 19.95 | 21.66 | 21.85 |
| Feb. 5th | 31.2 | 21.2 | 20.07 | 24.16 | 22.13 | 19.2 | 22.91 | 21.41 |
| 15th | 39.27 | 20.27 | 20.04 | 26.53 | 24.27 | 23.27 | 23.98 | 23.84 |
| 25th | 25.51 | 15.51 | 15.91 | 18.98 | 33.54 | 23.51 | 23.22 | 26.76 |
| Mar. 5th | 22.95 | 18.95 | 15.86 | 19.25 | 20.54 | 12.95 | 22.66 | 18.72 |
| 15th | 17.55 | 30.49 | 21.76 | 23.27 | 23.38 | 42.04 | 19.29 | 28.24 |
| 25th | 10.56 | 23.56 | 24.96 | 19.69 | 12.6 | 24.45 | 31.92 | 22.99 |
| Apr. 5th | 12.52 | 18.8 | 24.47 | 18.6 | 10.6 | 42.24 | 26.6 | 26.48 |
| 15th | 13.85 | 28.24 | 26.52 | 22.87 | 15.12 | 32.49 | 26.82 | 24.81 |
| 25th | 11.33 | 19.84 | 26.63 | 19.27 | 14.11 | 21.56 | 28.6 | 21.42 |
| May 5th | 8.82 | 14.11 | 22.04 | 14.99 | 12.82 | 23.8 | 26.16 | 20.93 |
| General mean/season. | 21.89 | 21.53 | 18.22 | | 20.24 | 23.71 | 19.73 | |

### 3.5. The reduction of *Empoasca discipiens* Pest population (%)

The results regarding the effectiveness of alternative pesticides combined with fertilizer application in controlling *Empoasca decipiens* Paoli infesting Calendula officinalis during both seasons are summarized in Figure 5. Applying all types of fertilizer with a pesticide rotation every 10 days proved effective in preventing pest resistance, as the continuous change helped maintain efficacy without significant decline. Additionally, the greatest reduction of *E. decipiens* was observed with NPK+PGPR, which was 12.79% and 13.06% higher than the reduction seen with NPK.

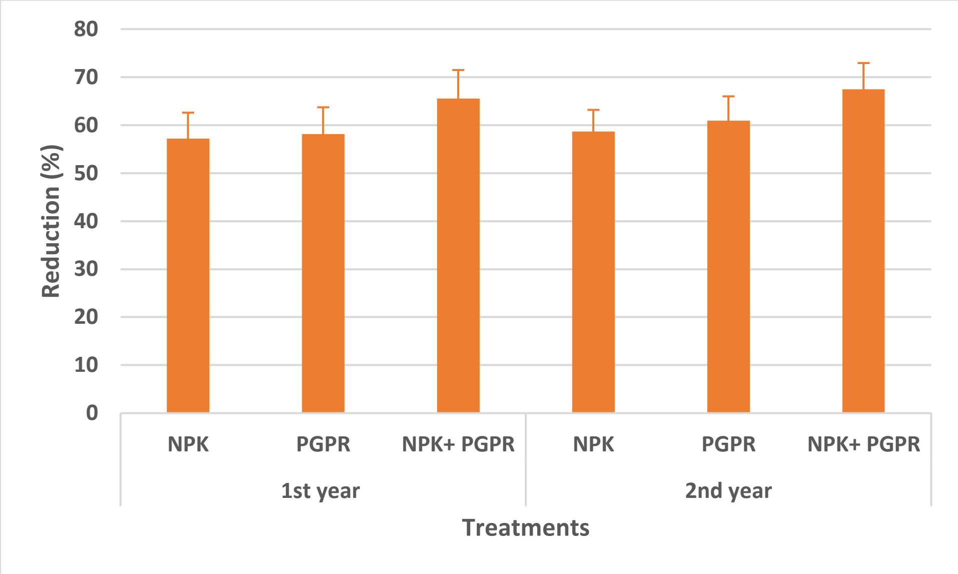



**Figure 5. Reduction percentage of the *Empoasca discipiens* Pest population. Data are presented as means ± SD.**

### 3.6. Phytoplasma percentage

The percentage of phytoplasma found on calendula plants during the two consecutive growing seasons of 2022–2023 and 2023–2024 is examined in detail in Figures 6 and 7. As seen in Figure 6, the data presented that the highest general mean of phytoplasma percentage observed was under the application of NPK, whilst the lowest general mean was recorded under the application of NPK + PGPR in both seasons, highlighting the synergistic effect of combining mineral fertilization with beneficial bacteria. Moreover, the application of fertilizer, especially the combination of NPK+PGPR, reduced the phytoplasma percentage. In both seasons, the percentage of phytoplasma was decreased by 82.96% and 83.11%, respectively, when using the alternative pesticides compared to the control under the application of NPK+PGPR.

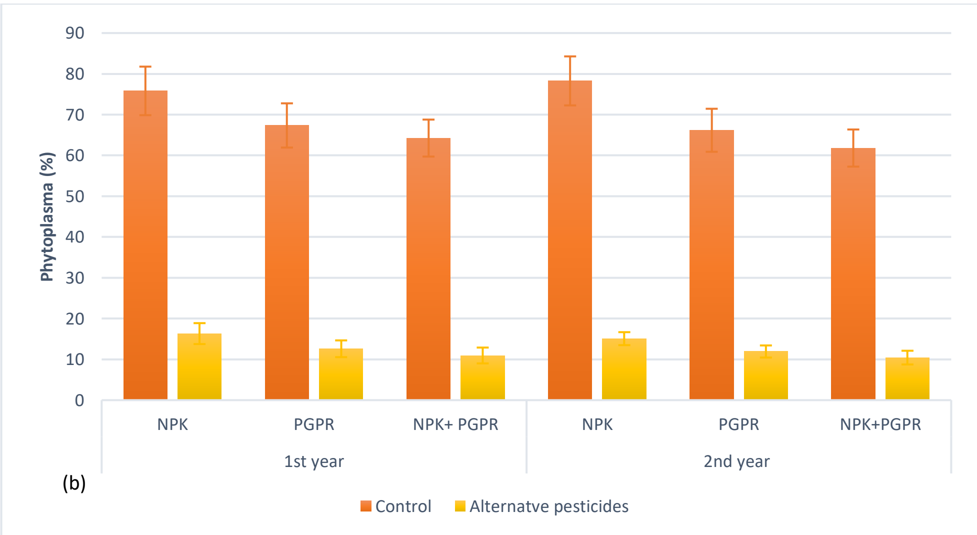



**Figure 6. The percentage of phytoplasma on Calendula during the 2022/2023 and 2023/2024 seasons. Data are presented as means ± SD.**


A- Plants and flowers infestation with phytoplasma

B- Plants and flowers are normal, not an infestation.

**Figure 7. Infestation of phytoplasma on plants and flowers.**

## 5. Discussion

Many researchers have reported on the ongoing global study into biofertilization as an alternative to chemical fertilization in medicinal and aromatic plants [45–48]. The current study found that using alternative pesticide treatments with NPK elements and PGPR significantly improved the morphological characteristics of calendula plants, leading to improved flowering traits (Figure, 2). These results may be due to fertilizers providing essential nutrients, aiding in biofertilization, stress management, and indirectly mitigating disease impacts. These results are in agreement with those of [49–52], who found that PGPR can improve plant growth, nutrient cycles, stress resilience, and promote abiotic stress tolerance. It also modulates hormonal and nutritional equilibrium, stimulates resistance to pathogens, and solubilizes nutrients for better uptake [16]. Several studies have reported the positive effect of PGPR on crop production; Abdallah *et al*. [53]; El-Serafy and El-Sheshtawy [54] on fennel, Ghatas and Mohamed [55] on *Oenothera biennis* L., and Fatmi [56], Aslam et al. [57] on mung bean, and Eissa *et al*. [58] on *Iris tingitan*a cv. Wedgewood. Moreover, many studies evaluated various pest control methods, revealing significant differences in environmental impact and effectiveness. For example, sulfur 30% Liquid is effective in managing pests like alfalfa weevil and *pistachio psylla*, with efficacy rates of up to 87.2%. Brave A M and KZ Oil may pose environmental hazards. Also, Potassium agricultural soap and Top Perfect are eco-friendly alternatives, but their efficacy data is limited [29,30,33].

Furthermore, the data presented in Figure (3) revealed that applying alternative pesticide treatments, alongside NPK and plant growth-promoting bacteria (PGPR), significantly enhances the flower yield and quality of *Calendula officinalis*. Also, Alternative pesticides demonstrated efficacy against various pests while being less harmful to non-target organisms. This synergistic approach optimizes nutrient uptake and promotes plant health, leading to improved flowering characteristics. These results were in harmony with those obtained by Shasidhara & Gopinath [59], who reported that the application of bioinoculants such as Azotobacter at 200 g/ha and VAM at 15.6 g/plant has been shown to enhance flowering duration and flower quality. These bioinoculants can substitute a portion of chemical fertilizers, maintaining yield while improving cost-effectiveness. The quality of flowers, including essential oil content and flavonoid levels, was positively influenced by applying bio-organic fertilizers and bio-stimulants. The highest concentrations of beneficial compounds, such as anthocyanins and total phenolics, were achieved with specific combinations of fertilizers and bio-stimulants [60]. Also, Zaferanchi *et al*. [61] found that the combination of NPK and PGPR has significantly increased calendula flower yield.

The obtained results of both seasons of Calendula plants illustrated in Figure (4) revealed that the use of Top Perfect 82% EC along with metallic compounds and the fertilization of NPK + PGPR has been found to significantly enhance overall plant biochemicals. Increased total chlorophyll levels in leaves, which is crucial for photosynthesis. Higher concentrations of beta carotenoids and total flavonoids in dry ray flowers contribute to nutritional and medicinal value. Improved nutrient content, including nitrogen, phosphorus, and potassium (NPK) percentages, and total carbohydrates in leaves, indicating better energy storage and plant health. These results are owing to the use of micronutrients that play an important role in the representation of critical auxins that increase cell division and increase the content of chlorophyll in the leaf. The results align with previous studies conducted by Hashem [62], highlighting fertilizers' positive effects on marigold plants. Additionally, the findings are consistent with those reported by

Abou-Sreea and Yassen [63], Jan *et al*. [64], Fatmi [56], Eissa *et al.* [58], Bohinc *et al*. [24], Al-Kuzaey et al. [65] , and Abdou et al. [66]

**6. Conclusion**

In brief, the results suggest that using the alternative pesticides Giskanim 1% EC, Sulfur 30% Liquid, Brave A M 6% SL, KZ oil 95% EC, Potassium agricultural soap, and Top Perfect 82% EC, applied every 10 days in rotation, combined with NPK plus PGPR, had a significant positive impact on Calendula plants. The combined application improved growth performance, flowering traits, and chemical constituents such as total chlorophyll in leaves, total carotenoids, total flavonoids in dry ray flowers, and the percentages of nitrogen, phosphorus, and potassium, compared to other treatments. Additionally, the treatment's ability to reduce pest populations underscores its potential as a useful tool in sustainable agriculture. Future research could further investigate the long-term effects and optimal application strategies to maximize these benefits.

**7. Author contributions**

Conceptualization, T.E.E., A.A., and O.M.I.; methodology, A.A., T.E.E., O.M.I.; software, T.E.E., and O.M.I.; formal analysis, O.M.I., and T.E.E.; investigation, T.E.E.; resources, O.M.I, A.A., and T.E.E.; writing—original draft preparation, A.A., T.E.E., and O.M.I.; writing—review and editing, A.A., T.E.E., and O.M.I. All authors provided critical feedback and helped shape the research, analysis, and manuscript. Also, all authors discussed the results and contributed to the final manuscript. All authors read and approved the final manuscript.

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