Agriculture Association of Textile Chemical and Critical Reviews Journal (2025) 73-78

AATCC Review

22 February 2025: Received
17 April 2025: Revised
24 April 2025: Accepted
27 May 2025: Available Online

https://aatcc.peerjournals.net/

Original Research Article

Open Access

Effect of bioagents on physiological and biochemical parameters in African Marigold (*Tagetes erecta* L.)under heat stress conditions

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ABSTRACT

African marigold (Tagetes erecta L.) is an important commercial flower crop, widely cultivated for loose flower production throughout the year. It serves as a lucrative cash crop, providing farmers with high returns. Farmers grow a range of marigold, especially African marigold varieties for different seasons; however, high temperatures limit marigold production during the summer season particularly in Northern India. An investigation was conducted to analyse the influence of bioagents (Anabaena torulosa, Pseudomonas nitroreducens, Providencia spp., and Trichoderma viride) on the physiological and biochemical responses of three marigold varieties i.e. 'Bidhan Marigold-1', 'Bidhan Marigold-2', and 'Punjab Gainda No.1' under open field heat stress conditions. The results showed that bioagent treatments mitigated heat-induced cell injury, as evidenced by a reduction in electrolyte leakage, and significantly enhanced gas exchange parameters such as photosynthetic rate, stomatal conductance, and transpiration rate compared to the control in all marigold varieties. Additionally, application of bioagents significantly increased the levels of cellular metabolites, including sugars, phenols, and proteins in the leaves, compared to untreated plants. The findings revealed that, among the three marigold varieties, 'Punjab Gainda No.1' exhibited the highest heat resistance, followed by 'Bidhan Marigold-2' and 'Bidhan Marigold-1'. Treatment with Trichoderma viride (through both soil drenching and foliar application) and Pseudomonas nitroreducens (via soil drenching) proved to be an effective strategy for improving marigold growth and yield under heat stress conditions, thus offering farmers a potential means of sustaining their livelihood in challenging environmental conditions.

Keywords: *African marigold, Heat stress, Bioagents, Trichoderma viride, Pseudomonas nitroreducens, Physiological, Biochemical parameters*

INTRODUCTION

African marigold (*Tagetes erecta* L.) belongs to the family Asteraceae and is one of the most important commercial flower crops known, widely cultivated for loose flower production [1]. It is a common traditional flower in India for its cultural, economic, and ecological benefits. Culturally, they play a vital role in festivals, weddings, and religious rituals, symbolizing prosperity [2]. Economically, marigold serves as a lucrative cash crop, offering farmers high returns and generating employment, particularly for women in rural areas [3]. The flowers are rich source of carotenoids [4] and serve as a potential leader in loose flower export. The growing demand for marigold in both domestic and international markets presents significant export potential, contributing further to the agricultural economy [5]. Farmers grow a range of marigold, especially African marigold varieties for different seasons; however, high temperatures limit marigold production during the summer season particularly in Northern India.

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DOI: https://doi.org/10.21276/AATCCReview.2025.13.03.73

African marigold, experiences heat stress when exposed to prolonged high temperatures, typically above 30-35°C, especially during critical growth stages such as vegetative and flowering stages [6]. Heat stress affects plant health and flower development, resulting in lower bloom count and also affects flower quality, shelf lifewhich leads to a decrease in market value.

Heat stress, which refers to exposure to temperatures that exceed the tolerance range of the corresponding species, is an agricultural problem for a wide range of plants across the world [7],[8]. Heat stress may cause a series of negative effects on the growth, metabolism and productivity of plants, including reduced germination percentage, abnormal seedlings, poor seedling vigour, and increased sterility [9],[10]. Either transitory or sustained high temperature can cause morpho-anatomical, phenological, physiological and biochemical responses in plants [11],[12],[13],[14]. Physiological responses of plant to heat stress are often associated with cell membrane thermostability, photosynthesis, accumulation of compatible osmolytes, water relations, hormonal changes, assimilate partitioning, and secondary metabolites [15],[16]. Biochemical responses of high temperature in plants lead to protein denaturation; as a result, soluble protein content gets reduced in untreated plants.

Phenolic compounds, with their structural diversity, regulate various physiological functions during growth and development and are involved in plant defence mechanisms. Sugars also play a vital role in scavenging reactive oxygen species and contribute to enhanced membrane stabilization [17].

In recent years, the application of bioagents-beneficial microorganisms such as mycorrhizal fungi and plant growth-promoting rhizobacteria (PGPR) has emerged as a promising, alternative, eco-friendly approach for the improvement of agricultural crop production and to enhance crop resilience under heat stress conditions [18],[19]. In the last couple of decades, a number of different researchers reported the use of plant growth-promoting bacteria (PGPB) for enhancing tolerance to heat stress in plants such as sorghum [20], chickpea [21], wheat [22], and tomato [23]. These bioagents improve plant health by facilitating nutrient absorption, particularly mineral phosphorus [24],[25], enhancing root development, and improving physiological responses such as water use efficiency [26]. Furthermore, bioagents induce critical biochemical changes that mitigate the damaging effects of heat stress. PGPR produce antioxidants that enhance the abscisic acid (ABA) accumulation and degradation of reactive oxygen species [11],[27]. By enhancing the plant's defence mechanisms [28], bioagents can improve stress tolerance and promote recovery from heat-induced damage. This study aims to explore the physiological and biochemical changes that occur in African marigold in response to the application of bioagents under heat stress, contributing essential knowledge for developing resilient agricultural practices.

MATERIALS AND METHODS

Planting material

Three cultivated varieties of African marigold *i.e.*'Bidhan Marigold-1' (BM-1), 'Bidhan Marigold-2' (BM-2) and 'Punjab Gainda No.1' (PG-1), were selected for experimentation.

Experimental site

The experiment was carried out for two years *i.e.* February to July, 2023, and 2024 Research Farm, Division of Floriculture and Landscaping (latitude of 28°40′N, longitude of 77°12′E) of Indian Council of Agricultural Research - Indian Agricultural Research Institute, New Delhi, India. Healthy seedlings 25-30 days old were transplanted into the main experimental field with a plot size of 1.3 m x 1.3 m at a density of 12 plants/m^2.

Details of bioagents and inoculation treatments

All the bioagents used in this investigation are procured from the Division of Microbiology, ICAR – Indian Agricultural Research Institute, New Delhi and their details are given in earlier investigations [29],[30]. Formulations of the selected microorganisms prepared using paddy straw compost and vermiculite as carrier (1:1) were used to prepare aqueous extracts as foliar and drench agents. A dose of 5 ml per plant was given at 3 stages - Transplanting, vegetative and flower bud initiation stages. All the data were recorded at peak temperature regime during summer, with the plants in full bloom stage.The treatments included: Control (water - foliar (T_1) and soil drench (T_2)); *Anabaena torulosa* formulation (foliar (T_3) and soil drench (T_4)); *Pseudomomas nitroreducens* formulation (foliar (T_5) and soil drench (T_6)); *Providencia spp.*formulation (foliar (T_7) and soil drench (T_8)); *Trichoderma viride* formulation (foliar (T_9) and soil drench (T_{10})). All the treatments were taken in triplicate using randomized block design (RBD) with factorial concept.

Estimation of growth and physiological parameters

Flower fresh weight: Fully opened flowers from plants in each treatment were weighed immediately after harvesting, and the average weight was expressed in grams.

Flower dry weight: The freshly weighed flowers were dried in a hot air oven at 65°C, till constant weight was obtained. The average value was recorded as the dry weight of flower, expressed in grams.

Electrolyte leakage: Ion leakage was determined by recording the electrical conductivity of leaf leachates in double-distilled water at 40°C and 100°C.Fully matured leaves were taken and made into uniform sized piecesof 0.1 g, then placed in test tubes containing 10 ml of double-distilled water in two sets: one set was maintained in a water bath at a constant temperature of 40°C for 30 minutes, while the other set was immersed in a boiling water bath at 100°C for 15 minutes. After the specified duration at each temperature, the respective conductivities were measured and denoted as C_1 for the 40°C sample and C_2 for the 100°C sample, using a conductivity meter [31],[32]. Electrolyte leakage was calculated by using the formula

- Electrolyte leakage (%) = $(C_1 / C_2) \times 100$

Gas exchange parameters: Photosynthetic rate (A), transpiration rate (E) and stomatal conductance (gs) were measured on fully expanded flag leaves using portable infra-red gas analyzer (IRGA) (Portable Photosynthesis System), Li - COR 6400 at full blooming stage [33].The photosynthetic rate was determined at different growth stages between 10:00 and 11.30 am by providing artificial light source of 1,000 μmol(photon) $m^{-2}s^{-1}$. Expressed as Photosynthetic rate (A) (μmol (CO_2) $m^{-2}s^{-1}$), stomatal conductance (gs) (mmol(H_2O) $m^{-2}s^{-1}$), and transpiration rate (E) expressed as (mmol(H_2O) $m^{-2}s^{-1}$).

Estimation of biochemical parameters

- Soluble protein content was estimated in fully matured leaf samples using the Bradford reagent method [34] with some modifications.
- Total phenol content in the leaf samples was measured following the protocol given by [33] using Folin-Ciocalteau reagent with some modifications.
- For the estimation of total soluble sugars, the method of [16] was followed with some modifications.

RESULTS AND DISCUSSION

Effect of bioagents on growth and physiological parameters in marigold under open field heat stress conditions

Fresh flower and dry weight

The data presented in Fig 1 and 2 indicates thatthe variety 'Punjab Gainda No.1' recorded the maximum fresh weight (4.56 g) on an average, succeeded by 'Bidhan Marigold-2'(4.25 g) and minimum in 'Bidhan Marigold-1' at 3.53 g. The flower fresh weight ranges from 3.11 to 5.28 g in case of treatments, highest was recorded with treatment *Trichoderma viride* formulation as soil drench (T_{10}) in 'Punjab Gainda No.1'and lowest with water as foliar application (T_1)of 'Bidhan Marigold-1'. From the Fig. 2, it was evident that the variety 'Punjab Gainda No.1'exhibited the maximum dry weight (0.84 g), followed by 'Bidhan Marigold-2'(0.80 g) and lowest in 'Bidhan Marigold-1' (0.51 g).The dry weight of flower ranges from 0.45 to 1.02 g, with the highest value exhibited in *Pseudomomas nitroreducens* formulation as soil drench (T_6) in'Punjab Gainda No.1'and lowest in water as soil drench (T_2) in 'Bidhan Marigold-1'. Microbial inoculants brought significant enhancement in weights which show that microbial activity modulates, directly/indirectly, the accumulation of photosynthates in plant systems, in *Mathiola incana,* a cut flower in accordance with [35].

Electrolyte leakage

Analysing the data from Fig 3 highlights significant variations related to electrolyte leakage. Specifically, the 'Punjab Gainda No.1'variety exhibited the lowest at 43.27%, followed by 'Bidhan Marigold-2'at 46.82%while 'Bidhan Marigold-1' recorded the highest electrolyte leakage at 50.15% among the varieties. In termsof treatments, *Trichoderma viride* formulation as foliar application (T_9) in'Punjab Gainda No.1'recorded minimum electrolyte leakage with 35.17% followed by *Anabaena torulosa* formulation as soil drench (T_4)at 39.88% in 'Bidhan Marigold-2'and treatment *Trichoderma viride* formulation as soil drench (T_{10}) at 45.12% in 'Bidhan Marigold-1'. Conversely, maximum electrolyte leakage was observed in water as soil drench (T_2) at 49.70% for 'Punjab Gainda No.1'and water as foliar application (T_1) at 52.91%, 56.86% for 'Bidhan Marigold-2', 'Bidhan Marigold-1' respectively. *Trichoderma* spp. played a pivotal role in alleviation of heat stress by regulating the production of reactive oxygen species and protecting the plant cells from oxidative damage, which reflects the stability of the cell membrane [36]. This is in agreement with [37] in tomato. Data in the study clearly indicates that the electrolyte leakage of 'Punjab Gainda No.1'was much lower under high temperature, suggesting that its membrane structure remains relatively stable under heat stress.

Gas –Exchange Parameters

Photosynthetic Rate

A marked increase in the photosynthetic rate (A) was observed in all the varieties, 'Punjab Gainda No.1'exhibited the highest photosynthetic rate, averaging 3.40 µmol $m^{-2}s^{-1}$ followed by 'Bidhan Marigold-2' with 3.02 µmol $m^{-2}s^{-1}$ and lowest was observed in 'Bidhan Marigold-1'with 2.82 µmol $m^{-2}s^{-1}$. Among the treatments, the highest photosynthetic rate was recorded in plants subjected to treatment *Trichoderma viride* formulation as soil drench (T_{10}) at 4.20 µmol $m^{-2}s^{-1}$, *Pseudomomas nitroreducens* formulation as foliar application (T_5) at 3.76 µmol $m^{-2}s^{-1}$ and *Trichoderma viride* formulation as foliar application (T_9) at3.45 µmol $m^{-2}s^{-1}$ for 'Punjab Gainda No.1', 'Bidhan Marigold-2' & 'Bidhan Marigold-1' varieties, respectively. In contrast, the lowest rates were recorded in water as foliar application (T_1) at 2.2 µmol $m^{-2}s^{-1}$, 2.01 µmol $m^{-2}s^{-1}$ for 'Punjab Gainda No.1', 'Bidhan Marigold-2' respectively and water as soil drench (T_2) at1.91 µmol $m^{-2}s^{-1}$ for 'Bidhan Marigold-1' (Fig 4) under heat stress conditions.

Transpiration Rate

Data regarding transpiration rate (E) of three varieties as shown in Fig 5, which indicates that 'Punjab Gainda No.1'had more transpiration rate (1.04mmol $m^{-2}s^{-1}$) on average, compared to 'Bidhan Marigold-2'(0.86 mmol $m^{-2}s^{-1}$) and 'Bidhan Marigold-1' (0.64 mmol $m^{-2}s^{-1}$). In relation to treatments, *Anabaena torulosa* formulation as soil drench (T_4) showed maximum transpiration rateat 1.37 mmol $m^{-2}s^{-1}$ in 'Punjab Gainda No.1' followed by *Pseudomomas nitroreducens* formulation as soil drench (T_6) at1.20 mmol $m^{-2}s^{-1}$ in 'Bidhan Marigold-2', and *Trichoderma viride* formulation as soil drench (T_{10}) at 0.95 mmol $m^{-2}s^{-1}$ in 'Bidhan Marigold-1'. In comparison, minimum transpiration rate was noticed in water as foliar application (T_1) at 0.71 mmol $m^{-2}s^{-1}$,0.51 mmol $m^{-2}s^{-1}$ and 0.37 mmol $m^{-2}s^{-1}$ for 'Punjab Gainda No.1','Bidhan Marigold-2', and 'Bidhan Marigold-1'respectively.

Stomatal Conductance

Results regarding stomatal conductance (gs) shown in Fig 6 indicates that maximum stomatal conductance was recorded in 'Punjab Gainda No.1'at 0.061 mmol $m^{-2}s^{-1}$ followed by 'Bidhan Marigold-2'at 0.045 mmol $m^{-2}s^{-1}$&'Bidhan Marigold-1' (0.033 mmol $m^{-2}s^{-1}$) respectively. In terms of treatments, the highest stomatal conductance was recorded in the treatments *Trichoderma viride* formulation as foliar application (T_9) at 0.082 mmol $m^{-2}s^{-1}$ for 'Punjab Gainda No.1',*Trichoderma viride* formulation as soil drench (T_{10}) at 0.072 mmol $m^{-2}s^{-1}$ for 'Bidhan Marigold-2', followed by *Pseudomomas nitroreducens* formulation as soil drench (T_6) at 0.053 mmol$m^{-2}s^{-1}$ for 'Bidhan Marigold-1'. Conversely, the treatment water as foliar application (T_1) recorded lowest stomatal conductance at 0.024 mmol $m^{-2}s^{-1}$, 0.016 mmol $m^{-2}s^{-1}$ for 'Bidhan Marigold-2', 'Bidhan Marigold-1' respectively, while water as soil drench (T_2) showed least at 0.02 mmol $m^{-2}s^{-1}$ for 'Punjab Gainda No.1'.

Trichoderma spp. showed positive impact on gas exchange parameters by showing better CO_2 influx supports a higher photosynthetic rate under *Trichoderma* treatment. It was evident in rice plants treated with *Trichoderma* spp., significantly stimulated the photosynthetic rate, stomatal conductance and water use efficiency [36],[21]. *Trichoderma* spp.showed positive impact by enhancing the plant's ability to retain water by promoting root growth and water uptake, thus reducing the heat stress-induced decline in stomatal conductance. With the production of antioxidants, oxidative stress was reduced. This resulted in lowering of stomatal closure caused by heat stress, inturn showed higher stomatal conductance and transpiration rate. As a nitrogen-fixer, *Anabaena* spp.*i.e.*cyanobacterium improves overall plant health and root development. It enhances the plant's antioxidant enzyme activities, reducing oxidative damage to guard cells under heat stress in accordance with [38]. It also produces extracellular polysaccharides that improve soil structure, enhancing water retention around the root zone enabling better water uptake, indirectly supporting transpiration under heat stress [29][36] in rice.

Biochemical Parameters

Soluble protein content

Examining the data extracted from Fig 7 regarding soluble protein content unveils that the variety 'Punjab Gainda No.1'displayed the highest soluble protein content at 7.22 mg/g fwt., followed by 'Bidhan Marigold-2'at 6.37 mg/g fwt., while 'Bidhan Marigold-1' exhibited the lowest at 4.80 mg/g fwt among the varieties. Regarding treatments, the maximum soluble protein content resulted from the treatment *Pseudomomas nitroreducens* formulation as soil drench (T_6) with 8.66 mg/g fwt. at par with *Trichoderma viride* formulation as soil drench (T_{10}) at 8.51 mg/g fwt. in 'Punjab Gainda No.1' followed by *Pseudomomas nitroreducens* formulation as foliar application (T_5) in 'Bidhan Marigold-2'at 7.82 mg/g fwt and *Providencia spp.*formulation as soil drench (T_8) in 'Bidhan Marigold-1' at 5.81 mg/g fwt. However, minimum soluble protein content was observed in the treatment water as soil drench (T_2) at 5.35mg/g fwt., 4.69 mg/g fwt., and 3.36 mg/g fwt for 'Punjab Gainda No.1','Bidhan Marigold-2'and 'Bidhan Marigold-1' respectively. *Pseudomonas* spp. strain AKMP7 improves thermal tolerance by lowering the levels of reactive oxygen species which can damage chlorophyll, protein, DNA, and lipids thus affecting plant metabolism and limiting growth and yieldin wheat [32]. This is in agreement with the report of the accumulation of cellular metabolites subjected to elevated temperature in sorghum seedlings [20].

Total phenol content

Markedly, 'Bidhan Marigold-2'variety showed the highest total phenol content at 106.57 µg/g fwt., followed by 'Punjab Gainda No.1'at 98.10 µg/g fwt., while the variety 'Bidhan Marigold-1' exhibited the lowest at 78.94 µg/g fwt. Among the treatments, maximum total phenol content was recorded with *Trichoderma viride* formulation as soil drench (T_{10}) at 133.38 µg/g fwt. in 'Bidhan Marigold-2' followed by *Pseudomomas nitroreducens* formulation as foliar application (T_5) at 124.10 µg/g fwt., in 'Punjab Gainda No.1' and *Providencia* spp.formulation as foliar application (T_7) at 102.07 µg/g fwt. in 'Bidhan Marigold-2'. Whereas, the treatment water as foliar application (T_1) recorded minimum total phenol content at 55.61µg/g fwt., 81.18µg/g fwt.for 'Bidhan Marigold-1','Bidhan Marigold-2' respectively. The treatment water as soil drench (T_2) showed the lowest at 76.10µg/g fwt. for 'Punjab Gainda No.1'(Fig 8). In the present study, there was aconsiderable increase in total phenol content in leaf samples from treated one as compared to the untreated, which clearly indicates the efficiency of *Trichoderma* spp. in alleviating thermal stress. A significant increase in total phenolic content at higher temperature was reported by [39] in watermelon plants. [40] observed that seed treatment with *T. Viride* enhanced the total phenol content in chickpea plants.

Total soluble sugars

Significant variations are evident in the data extracted from Fig 9, specifically related to the total soluble sugars. Among the three varieties, 'Bidhan Marigold-2'stands out with the highest recorded total soluble sugars content at 225.21 mg/g fwt., followed by 'Punjab Gainda No.1'at 209.60 µg/g fwt., while 'Bidhan Marigold-1' exhibits the lowest at 187.84 mg/g fwt. In terms of treatments, the maximum total soluble sugars content (272.05 mg/g fwt.) results from the treatment *Trichoderma viride* formulation asfoliar application (T_9) in 'Bidhan Marigold-2' succeeded by *Trichoderma viride* formulation assoil drench (T_{10}) at 260.80 mg/g fwt.in 'Punjab Gainda No.1' and *Anabaena torulosa* formulation as soil drench (T_4) at 225.44 mg/g fwt.in 'Bidhan Marigold-1' while the minimum recorded in the treatment water as foliar application (T_1) at 164.99 mg/g fwt., 141.72 mg/g fwt.and 119.70 mg/g fwt.for 'Bidhan Marigold-2', 'Punjab Gainda No.1' and 'Bidhan Marigold-1' respectively. Accumulation of total soluble sugars under heat stress has been implicated in the establishment and maintenance of thermotolerance in accordance with [41],[42] in arabidopsis plants.

CONCLUSION

Application of *Trichoderma viride* (soil drench and foliar respectively), *Pseudomonas nitroreducens* (soil drench) could be an effective approach in African marigold varieties 'Punjab Gainda No.1' followed by 'Bidhan Marigold-2' under heat stress. The relationship between physiological and biochemical responsesand the degree of susceptibility to the heat stressstudied is evident in the marigold varieties; 'Punjab Gainda No.1' in particular, clearly indicates that the *T. viride* improves heat stress tolerance in plants by regulating the production of reactive oxygen species; protecting the plantcells from oxidative damage in turn elevating the levels of cellular plant defence response. Thereby, it can befurther said that the use of these bioagents not only increase the crop regime toward higher temperature but also help the farmers to sustain their crop and its yield during the heat stress.

Compliance with Ethical Standards

Conflict of interest: The authors declare that they have no conflict of interest.

Acknowledgment

The author would like to thank Division of Floriculture and Landscaping, ICAR-IARI, New Delhi for providing materials and all the necessary resources required for conducting the research.

Author's contribution

Bhanu Murthy K C conducted the research experiment and analyzed the data; Sapna Panwar, RadhaPrasanna conceptualized the research and guided throughout the experiment; K.P Singh and Namita helped in main manuscript writing and forming tables; Vignesh Muthuswamy and Sudhir Kumar helped in data curation.

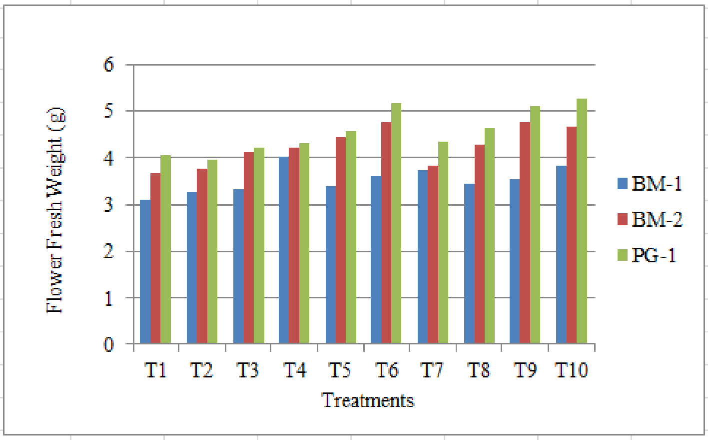

Fig 1. Effect of bioagents on flower fresh weight of African marigold varieties under open field heat stress condition

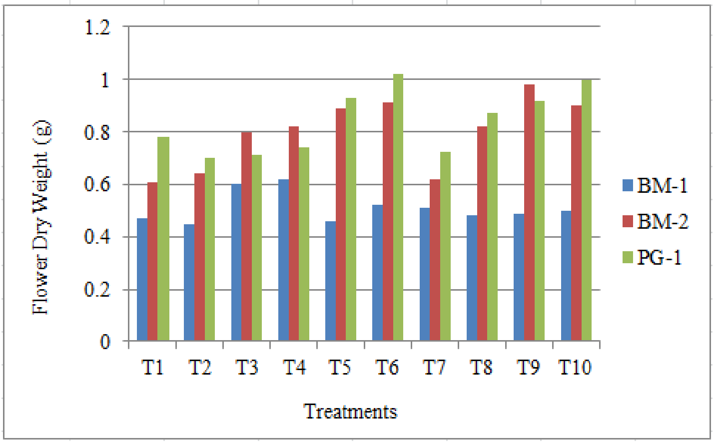

Fig 2. Effect of bioagents on flower dry weight of African marigold varieties under open field heat stress condition

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