SHST
Horticulture and Forestry
Society of Transylvania

Syropoulou A *et al.* (2022)
Notulae Scientia Biologicae
Volume 14, Issue 1, Article number 11220
DOI:10.15835/nsb14111220
Research Article

NSB
Notulae Scientia
Biologicae

Effects of organic and inorganic fertilization on growth and yield of *Physalis peruviana* L. crop under Mediterranean conditions

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Abstract

Physalis peruviana L. is an Andean Solanaceae fruit crop with great nutraceutical qualities, potential health benefits and adaptability to Mediterranean climates. In the current study, a first approach on the effect of organic and inorganic fertilization on *P. peruviana* crop under Mediterranean semi-arid conditions was performed. A field experiment was laid out according to a completely randomized design, with three replicates and three fertilization treatments [untreated (control), organic fertilization (biocyclic humus soil) and inorganic fertilization (inorganic fertilizer 40-0-0+14.5 SO_3)]. Phenological growth stages and their corresponded growing degree days were evaluated. In addition, some growth parameters, fruit yield and yield components were evaluated. The results indicated that the duration of phenological growth stages was in accordance with durations mentioned in tropical climate. The highest branches number per plant (24.4), leaf area per plant (1997.3 cm^2), fruit number per plant (41.52), fruit yield (7.51 t ha^{-1}) and average fruit weight (5.32 g) were found in inorganic fertilization plots, whereas the highest plant height (44.15 g) and fruit diameter (12.52 mm) were recorded under organic fertilization; however, the differences between the organic and inorganic fertilization were not statistically significant. In terms of dry weight per plant, there were significant differences among the fertilization treatment with the values obtained under inorganic fertilization (81.24 g). To sum up, *P. peruviana* showed satisfying adaptability under Mediterranean climate conditions and has great potential in becoming an alternative cultivation for small and medium producers of Mediterranean countries. In addition, the results indicated that organic fertilization (with biocyclic humus soil) should be considered as an alternative to inorganic fertilizers for *P. peruviana* production.

Keywords: biocyclic humus soil; fruit yield; growing degree days; growth parameters; nitrogen fertilization; yield components

Received: 12 Feb 2022. Received in revised form: 16 Mar 2022. Accepted: 17 Mar 2022. Published online: 18 Mar 2022.
From Volume 13, Issue 1, 2021, Notulae Scientia Biologicae journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

Introduction

Physalis peruviana L. (Solanaceae) is an herbaceous, semi-shrub, upright, and annual in temperate zones (perennial in tropical zones) plant, and can grow to 1 m. It is indigenous to South America Andes, mainly Peru, Colombia, and Ecuador (Muniz *et al.*, 2014). The cultivation of *P. peruviana* in South America can be traced back to the Inca Empire. This species has been grown in England since the end of the 18th century, and in South Africa from the early 19th century in the Cape Peninsula. *P. peruviana*, which was widely introduced in the 20th century, is now cultivated or grows wild in temperate and tropical regions worldwide. In general, *P. peruviana* can grow in a wide variety of soil and climatic conditions and is considered a fairly tolerant plant due to its adaptation to Mediterranean climates and to several soil types (Fischer *et al.*, 2011).

P. peruviana produces a small orange, berry-shaped, and sweet fruit that is high in provitamin A and ascorbic acid, as well as alkaloids, flavonoids, carotenoids, fructose, sucrose esters, polyphenols and bioactive compounds (Muniz *et al.*, 2014; Bertoncelli *et al.*, 2017). *P. peruviana* fruit is often consumed fresh giving an acid-sweet balance to fruit and vegetable salads (Puente *et al.*, 2011). At present, the fruit of *P. peruviana* is processed into a variety of products, including jams, juices and raisins (Ramadan and Moersel, 2007). It is used as an ornament in meals, salads, desserts, and cakes in European markets (Puente *et al.*, 2011; Bertoncelli *et al.*, 2017). *P. peruviana* has many therapeutic characteristics, including antispasmodic, diuretic, antiseptic, antidiabetic sedative, analgesic, aiding in optic nerve fortification, throat trouble relief, and elimination of intestinal parasites and amoeba (Cárdenas-Barboza *et al.*, 2021). Moreover, the fruit of *P. peruviana* is used empirically in Peruvian traditional medicine to cure cancer and other ailments such as hepatitis, asthma, malaria, and dermatitis; nevertheless, their properties have not been scientifically verified (Mayorga-Cubillos *et al.*, 2019). In addition, *P. peruviana* calyces (capsules) are widely utilized in traditional medicine for their anticancer, antibacterial, antipyretic, diuretic, and anti-inflammatory immunomodulatory effects (Puente *et al.*, 2011; Cárdenas-Barboza *et al.*, 2021).

Although *P. peruviana* is gaining popularity among producers, there is a lack of work and research results to guide them in terms of cultural practices, productivity, and economic aspects of production. Currently, fertilizer management in *P. peruviana* crop is based on tomato crop recommendations (Muniz *et al.*, 2014; Ariati *et al.*, 2017; Bertoncelli *et al.*, 2017). Only a few researchers have reported the use of organic compost in the cultivation of *P. peruviana* (Ariati *et al.*, 2017; de Souza *et al.*, 2021).

Crop nutrition is the most important component to consider in an agricultural production system since it directly affects crop performance, requiring soils with high nitrogen, potassium, calcium, and boron availability (Muniz *et al.*, 2014; Xin *et al.*, 2016; Bertoncelli *et al.*, 2017). However, in typical cropping systems, excessive use of fertilizers and chemical fertilizers, as well as poor soil management, result in water contamination (Xin *et al.*, 2016; Cihangir and Oktem, 2019). In order to reduce these environmental repercussions, the search for sustainable alternatives such as organic agriculture arises, as this approach is beneficial to crop productivity as well as life quality (Xin *et al.*, 2016; de Souza *et al.*, 2021).

Literature survey revealed that there was no information available concerning the performance of *P. peruviana* growth cultivated under Mediterranean semi-arid conditions and organic cropping system. As a result, the purpose of the present study aimed to investigate the effect of organic and inorganic fertilization on plant growth and yield of. *P. peruviana* in Mediterranean field conditions.

Materials and Methods

A *P. peruviana* crop was established in the organic experimental field of the Agricultural University of Athens (Latitude: 37°59′ N, Longitude: 23°42′ E, Altitude: 30 m above sea level) from April to August 2019. The soil was a clay loam (29.2% clay, 35.1% silt and 35.7% sand) with pH (1:1 H_2O) 7.38, nitrate-nitrogen (NO_3-N) 12.6 mg kg^{-1} soil, available phosphorus (P) 13.6 mg kg^{-1} soil, available potassium (K) 203 mg kg^{-1} soil, 15.83% $CaCO_3$ and 1.86% organic matter. The site was managed according to organic agricultural guidelines (EC 834/2007). Weather data (mean air temperature and precipitation) pertaining to the experimental period were recorded by the automatic weather station (Davis Vantage Pro2 Weather Station; Davis Instruments Corporation, California, USA) of the Agricultural University of Athens and are presented in Figure 1.

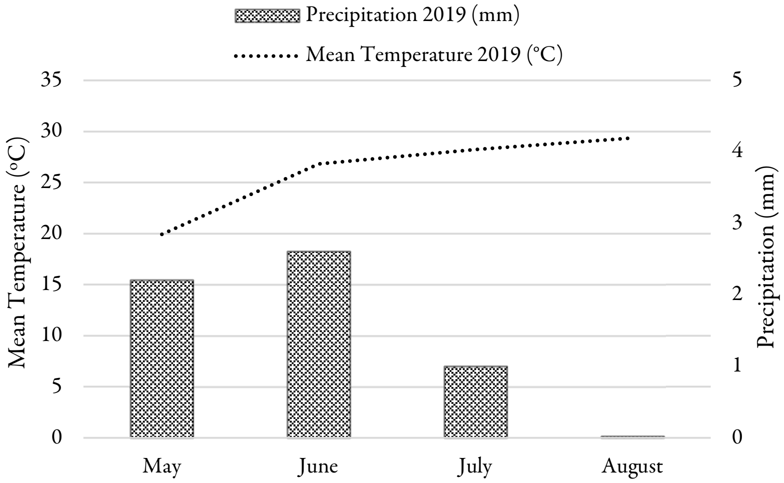

Figure 1. Meteorological data (mean temperature and precipitation during the growing period (May-August 2019)

Initially, five polystyrene floating trays with 198 cells per tray (17 cm^3 per cell) were filled with organic peat. On each individual cell, one seed of *P. peruviana* was sown. The polystyrene trays were then placed in a 250 L basin filled with water. In the basin, 2.5 L of organic water-soluble fertilizer (Fishfert, 2-4-0.5 and other trace elements; Humofert Co., Athens, Greece), as well as 25 g of organic enhancer (Trianum-P; Koppert BV, Berkel en Rodenrijs, The Netherlands) for plant protection were added. When the seedlings presented two pairs of true leaves with approximately 15 cm, which takes 25 days after sowing, they were transplanted to the final position in the field. The transplanting of the seedlings was done on 14th May 2019. The soil was prepared by mould-board ploughing at a depth of 0.25 m and the experiment was arranged in a completely randomized design (CRD) with three replications and three fertilization treatments: control-untreated, inorganic fertilization and organic fertilization with biocyclic humus soil. The plot size was 12.25 m^2 (3.5 m × 3.5 m). In each plot, 88 seedlings were transplanted at a spacing of 40 cm × 30 cm. One day before transplanting of *P. peruviana* 250 kg ha^{-1} of the inorganic fertilizer (Nutrimore Winner 40-0-0+14.5 SO_3, Gavriel Ltd.) was applied manually. The biocyclic humus soil was also applied by hand into the planting rows with 44 L in each, which is 4 L for each plant. Inorganic fertilizer and biocyclic humus soil were incorporated with the soil by harrowing. In addition, a drip irrigation system was also set up in the experimental field. The total quality of water applied during the cultivation period was 678 mm. Throughout the experimental periods, there was no incidence of pest or disease on P. *peruviana* crop. Weeds were controlled by hand-hoeing when needed.

The biocyclic humus soil, which was used in this experiment and sourced from Biocycle Vegan Company, is made entirely of plant materials, primarily by-products of olive oil mills. The raw materials consisted of 50% olive leaves, 30% olive pomace, 10% grape pomace, and 10% ripe humus soil. First, an aerobe composting process was carried out in rows 1.5 m high and 2.5 m wide. The raw materials were aerated and hydrated using a compost windrow turner. A ripe compost of substrate quality was obtained after 5 to 6 months of composting. A three-year ripening process was followed to convert the ripe compost into humus soil. The resulting material is beyond the substrate maturity and has a more soil-like structure that is suitable for direct planting. The biocyclic humus soil contained 2.8 g total nitrogen, 0.8 g P_2O_5 soluble in inorganic acids (total), 0.6 g total potassium, 7.6 units electrical conductivity (1:5) pH and 91.9 cation exchange capacity (C.E.C.)

meq Na per 100 g humus soil. It was certified in accordance with the Biocyclic Vegan Standard, which became a global standard in December 2017 and a full member of the IFOAM's Organic Family of Standards (Eisenbach *et al.*, 2019).

Plant height, number of branches per plant, leaf area per plant and dry weight per plant were determined on ten randomly selected plants from each plot at 80 days after transplanting (DAT). Dry weight was determined after drying for 48 h at 64°C. An automatic leaf area meter was used to calculate leaf area (Delta-T Devices Ltd., Burwell, Cambridge, UK). Moreover, fruit yield, fruit number per plant, average fruit weight and fruit diameter (without the capsule) were determined by plants derived from the middle sub-plot area (1 m^2) at fruit maturity on 30th August 2019 (128 DAT).

Finally, heat accumulation in growing degree days (GDDs) from transplanting until fruit maturity, summing the growing degree days in each time period evaluated (Pathak and Stoddard, 2018). Growing Degree Days (GDD) was calculated as:

$$GDD_i = \sum_i^n \left[\frac{Tmax + Tmin}{2}\right] - T_{base}$$

where, GDDi is the accumulated growing degree days, T_{min} and T_{max} are the minimum and maximum air temperature, respectively. T_{base} is *P. peruviana* base temperature (T_{base} = 6.29°C) (Salazar *et al.*, 2008).

The experimental data were checked for normality and subjected to statistical analysis using the SigmaPlot 12 statistical software (Systat Software Inc., San Jose, CA, USA) according to the completely randomized design (CRD). The differences between means were separated using Least Significance Difference (LSD) test. All comparisons were made at the 5% level of significance.

Results and Discussion

Thermal Time and Phenological Growth Stages

P. peruviana seedlings, consisted of 4-5 leaves each, were transplanted on 14th May 2019 (12.81 GDD; Table 1). After 556.23 GDD, plants entered fast development and leaf growth during the first half of June (vegetative stage). This stage lasted around one month from the first half of June to the first half of July. Flowering began around the first half of July after obtaining 1217.63 GDD (Figure 2a). Fruit formation was initiated during the first half of August and this stage received 1922.99 GDD. At this time period flowering and fruit formation were occurring simultaneously, proving the fact that *P. peruviana* has a tendency of indeterminate growth. Moreover, when the measurement of specific plant characteristics took place, fruit maturity was at a middle stage (fruit: yellow to orange) with a total obtainment of 2267.14 GDD.

Table 1. Accumulated Growing Degree Days (GDDs) from transplanting to fruit maturity and dates of basic phenological stages obtained during the growing cycle.

Phenological growth stages	Date	Days after transplanting (DAT)	GDDs (°C day)
Transplanting	14th May 2019	-	12.81
Vegetative	10th June 2019	27	556.23
Flowering	12nd July 2019	59	1217.63
Fruit formation	6th August 2019	104	1922.99
Fruit maturity	30th August 2019	128	2267.14

According to National Research Council (NRC) (1989), flowering of *P. peruviana* occurs 65-75 days after planting. Furthermore, the beginning of harvesting takes place 85-100 days after that. The results of the present study agree with the results above as flowering was initiated approximately 59-70 days after transplanting (Table 1). Moreover, 128 days after transplanting, fruit maturity was at a middle stage (fruit: yellow to orange) thus assuming that harvesting of ripe fruits could potentially start around the time period mentioned from NRC (1989). Miranda (2005) reported the duration of phenological stages of *P. peruviana* as a monoculture in Colombia. The flowering is initiated after 2 months of transplanting seedling of *P. peruviana* in final field position. In continuation with this stage, fruit formation begins 1 month after flowering and 1.5 months after fruits have formed occurs fruit maturity. Harvesting of fruits arises around 2.5 months after fruit formation. The duration of the phenological growth stages that were observed in this study are in accordance with Miranda (2005). Flowering stage of *P. peruviana* occurred around 2 months after the day of transplanting (Table 1). In addition, fruit formation took place approximately 1 month after flowering and a middle stage of fruit maturing was spotted on the day of measurement.


(A) (B)

Figure 2. *P. peruviana* at (A) flowering and (B) fruit formation stage, respectively

Growth, Yield and Yield Parameters of P. peruviana

The plant height was significantly affected (F=5.324, p= 0.0341) by the different fertilization treatments (Table 2). The highest mean plant height (44.15 cm) was observed in the organic treatment followed by the inorganic treatment (40.54 cm); however, the differences among these treatments were not statistically significant. Similarly, Ariati *et al.* (2016) found a slightly better response of organic fertilizer (poultry litter) on plant height of *P. peruviana* with issuing an average height of 180.9 cm in comparison with mineral fertilizer (167.7 cm). Although the results for plant height of the present study are according with the results of Ariati *et al.* (2016), the numerical differences observed between the plant heights may due to the fact that *P. peruviana* crop was cultivated in the region of Brazil in a tropical climate that favours the overall growth of this species, in contrast to the Mediterranean climate where *P. peruviana* only shows adaptability. De Souza *et al.* (2021) obtained different results at 45 days after transplanting (DAT) in *P. peruviana* crop with the highest plant height recorded with chemical fertilizer (28.66 cm) and the second highest on organic fertilizer that was contained in organic compost produced from cattle, goat manure, and tree pruning waste (27.38 cm). The results between inorganic and organic fertilization were not significantly different. This results difference could be explained by the fact that the organic fertilizer mentioned above was dissimilar with the one that was used in the present study.

Table 2. Plant height, number of branches per plant, leaf area per plant and dry weight per plant of *P. peruviana* as affected by fertilization

Fertilization	Plant height (cm)	Number of branches per plant	Leaf area per plant (cm^2)	Dry weight per plant (g)
Control	24.92 b	12.5 b	1317.6 b	57.63 c
Organic	44.15 a	23.9 a	1723.4 ab	74.32 b
Inorganic	40.54 a	24.4 a	1997.3 a	81.24 a
$F_{\text{Fertilization}}$	5.324*	7.327**	12.223*	9.463**

Different letters within a column indicate significant differences according to LSD test (p = 0.05). Significance levels: * p < 0.05; ** p < 0.01; *** p < 0.001; ns, not significant (p > 0.05).

The number of branches per plant is presented in Table 2. The effect of different fertilization regimes was found to be statistically significant (F= 7.327, p= 0.0097). Specifically, the highest number of branches (24.4) was achieved in plots fertilized with inorganic fertilizer followed by organic fertilization (23.9). Similar results were also recorded in tomato crops by several researchers (Mehla *et al.*, 2000; Bilalis *et al.*, 2018; Roussis *et al.*, 2019). Bertoncelli *et al.* (2017) also reported a significantly increased number of branches in the P. peruviana crop with the increase of nitrogen doses (0 to 350 kg N ha^{-1}), while this trait was not affected by phosphorus and potassium. The applied nutrients play a pivotal role in the assimilation of amino acids and nucleic acids, as well as the regulation of many metabolic processes, which increases photosynthetic efficiency (Roussis *et al.,* 2019). Increased soil fertility leads to increased availability of applied nutrients, particularly nitrogen, which promotes vigorous plant growth while limiting profuse branching and leaf production.

The analysis of variance revealed that leaf area per plant was actually affected by the different fertilization treatments (F= 12.223, p= 0.0232). In particular, the highest value (1997.3 cm^2) recorded in inorganic treatment, while the lowest value (1317.6 cm^2) obtained from the untreated (control) plot (Table 2). This is in accordance with previous studies in *P. peruviana,* confirming the positive response of this species to inorganic nitrogen fertilization (El-Tohamy *et al.*, 2009; Bertoncelli *et al.*, 2017). In general, higher nitrogen availability to plant result in greater leaf area, which leads to greater light absorption and further carbon fixation (Field and Mooney, 1986; Kakabouki *et al.*, 2018).

Regarding the dry weight per plant, there were statistically significant differences among fertilization regimes (F= 9.463, p= 0.0074) and the highest value (81.24 g) was found in inorganic fertilization treatment (Table 2). In an experiment with *P. peruviana*, inorganic nitrogen fertilization also positively influenced the total plant mass, reaching 472.50 g per plant with the dose of 200 kg N ha^{-1} and 274.25 g per plant in 50 kg N ha^{-1} dose (El-Tohamy *et al.*, 2009). Nitrogen fertilization increased vegetative growth and biomass accumulation since it increases photosynthate source capacity (Bilalis *et al.*, 2018).

According to the analysis of variance, the number of fruits per plant was significantly affected (F= 6.757, p= 0.0008) by different fertilization treatments (Table 3). The highest fruit number (37.53) obtained in the inorganic fertilization treatment followed by organic fertilization with biocyclic humus soil (37.53), while the lowest value (12.47) was observed in untreated (control) plants. Generally, the apparent deficiency of an adequate supply of plant-available nitrogen from organic fertilizers, caused by a slow rate of mineralization, leads crop yield and its components to be lower in fields treated with organic fertilizers than in those treated with inorganic fertilizers (Blatt, 1991).

Table 3. Fruit number per plant, fruit yield, average fruit weight and fruit diameter of *P. peruviana* as affected by fertilization

Fertilization	Fruit number per plant	Fruit yield ($t\ ha^{-1}$)	Average fruit weight (g)	Fruit diameter (mm)
Control	12.47 b	3.69 b	4.47 b	9.89 b
Organic	37.53 a	6.89 a	4.93 ab	12.52 a
Inorganic	41.52 a	7.51 a	5.32 a	12.03 a
$F_{Fertilization}$	6.757***	42.622*	24.214*	34.121**

Different letters within a column indicate significant differences according to LSD test ($p = 0.05$). Significance levels: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns, not significant ($p > 0.05$)

The fruit yield differed among the fertilization regimes (F= 42.622, p= 0.0387) with the highest value (7.51 t ha^{-1}) observed in the inorganic treatment followed by organic fertilization (6.89 t ha^{-1}). The lowest value (3.69 t ha^{-1}) was recorded in the untreated plots. The higher yield in inorganic fertilization is attributed to increased availability of nitrogen with elevated soil fertility levels as applied nutrients help in the vigorous growth of plants with an increased number of branches, flowers, and fruits. In an experiment developed by Bertoncelli *et al.* (2017) with *P. peruviana* plants, the authors obtained an increase in fruit yield per plant, as well as in the fruit mass, as a function of increase in nitrogen dose, a similar trend with the nitrogen availability observed in the current study. According to El-Tohamy *et al.* (2009), the increase in the number of fruits per plant, in *P. peruviana*, with an increase of available nitrogen is probably due to the fact that nitrogen is the element absorbed in greater quantity and with a fundamental importance for the growth and development of *P. peruviana* plants.

The results of the present study indicated that the effect of organic and inorganic fertilization on average fruit weight was statistically significant (F= 24.214, p= 0.0217). The highest average fruit weight (5.32 g) was observed in the inorganic treatment followed by the organic treatment (4.93 g). Bertoncelli *et al.* (2017) had confirmed that the average weight of *P. peruviana* fruits was increased with the elevation of the available nitrogen to the plant. In tomato crop, Bilalis *et al.* (2018) observed that the mean fruit weight was not influenced by fertilization; however, significant differences could be due to the variation in cultivars, soil type, temperature and precipitation during the cultivation period.

Fruit diameter (without the capsule) of *P. peruviana* was significantly affected by fertilization (F= 34.121, p= 0.0026) and the highest value (12.52 mm) observed in organic fertilization with biocyclic humus soil followed by inorganic fertilization (12.03 mm). These results are in line with those observed by Muniz *et al.* (2011), that when evaluating the effect of inorganic fertilizer (NPK 5-20-10) and organic (50% bovine manure compost and 50% swine) in *P. peruviana* obtained larger diameter fruit in plants fertilized with the organic fertilizer (21.14 mm with capsule) and the inorganic resulted in fruits with a mean diameter of 19.98 mm. On the contrary, Ariati *et al.* (2017), as they recorded an average fruit diameter (without the capsule) for *P. peruviana* of 18.75 mm with inorganic fertilizer, 18.30 mm with organic and 17.81 mm with control. In tomato plant, it was observed that this attribute is highly dependent on genetic factors linked to cultivars (Bilalis *et al.*, 2018).

Conclusions

The results of the current research study on *P. peruviana* confirmed that this species presents substantial adaptability to Mediterranean climate condition, as the durations of most phenological growth stages were similar with those mentioned in areas with tropical climate where this plant is indigenous. In addition, the current results also confirmed that growth, yield and yield parameters were affected by fertilization. Specifically, the highest branches number per plant, leaf area per plant, fruit number per plant, fruit yield and average fruit weight were found in inorganic fertilization plots, whereas the highest plant height and fruit diameter were recorded under organic fertilization; however, the differences between the organic and inorganic fertilization were not statistically significant. In terms of dry weight per plant, there were significant differences among the fertilization treatment with the values obtained under inorganic fertilization. To sum up, *P. peruviana* presents considerable adaptability to Mediterranean climates. It has great potential in becoming alternative cultivation for small and medium producers of Mediterranean countries. Moreover, the current study indicated that organic fertilization (with biocyclic humus soil) should be considered as an alternative to inorganic fertilizers for *P. peruviana* production. Further experimentation on this plant species is required regarding the appropriate cropping techniques in order to ensure its effective organic cultivation in Mediterranean areas.

Authors' Contributions

Conceptualization: I.K., D.B. (Dimitrios Bilalis); Data curation: A.S., I.R., I.K. and D.B. (Dimitrios Bilalis); Formal analysis: A.S., I.R., I.K., and D.B. (Dimitrios Bilalis); Investigation: A.S., I.R., S.K., V.K., I.K., A.M., A.F., D.B. (Dimitrios Beslemes) and D.B. (Dimitrios Bilalis); Methodology A.S., I.R., S.K., V.K., I.K., A.M., A.F., D.B. (Dimitrios Beslemes) and D.B. (Dimitrios Bilalis); Project administration: I.K. and D.B.; Resources: A.S., I.R., S.K., V.K., I.K., A.M., D.B. (Dimitrios Beslemes) and D.B. (Dimitrios Bilalis); Supervision: D.B. (Dimitrios Bilalis); Validation: A.S., I.R., S.K., V.K., I.K., A.M., A.F., D.B. (Dimitrios Beslemes) and D.B. (Dimitrios Bilalis); Visualization: I.K and D.B. (Dimitrios Bilalis); Writing - original draft: A.S., I.R., S.K., V.K., I.K. and D.B. (Dimitrios Bilalis); Writing - review and editing: A.S., I.R., I.K. and D.B. (Dimitrios Bilalis). All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

Acknowledgements

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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