Interactive effect of organic and mineral phosphorus on volatile compounds, morphology, and physiology of garlic

by Shahbaz Ali

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Abstract

Background: Phosphorus (P) is a macronutrient required by the plants for their normal growth and development. However, the impacts of different forms of P have been rarely tested on the morphology, physiology, and biochemical attributes of garlic (*Allium sativum* L.)

Methods: This study examined interactive effect of mineral-P (mP) as single super phosphate (SSP), and organic-P (oP) as farmyard manure (FYM) on morphological, physiological traits and volatile sulfur containing compounds of garlic. The treatments included T_0 = control, T_1 = 50 kg ha^{-1} mP, T_2 = 70 kg ha^{-1} mP, T_3 = 90 kg ha^{-1} mP, T_4 = 50 kg ha^{-1} mP + oP ≈ 50 kg ha^{-1}, T_5 = 70 kg ha^{-1} mP + oP ≈ 60 kg ha^{-1}, T_6 = 90 kg ha^{-1} mP + oP ≈ 30 kg ha^{-1}, and T_7 = 50 kg ha^{-1} mP + oP ≈ 90 kg ha^{-1}.

Results: The plant morphological and physiological traits as well as contents of the volatile sulfur containing compounds were significantly altered by the interactive effect of mP and oP. The garlic plants that were treated with T_7 generated the maximum bulb output (4382.96 kg ha^{-1}), followed by those fertilized with T_5, T_6, T_2, and T_4 which produced 4205.43, 4164.10, 3992.73, and 3916.30 kg ha^{-1}, respectively. The harvest was brought down to 3722.80 kg ha^{-1} in T_3, and it was just 3509.95 kg ha^{-1} in T_1. The response of volatile sulfur containing compounds in garlic leaves (detected by GC–MS and HPLC analysis) indicated that in T_7 enormously higher count of constituents in garlic leaves were determined which include aldehydes (3.254 μg/g), hydrocarbons(1.245 μg/g), esters (0.547 μg/g), acids (1.658 μg/g), sulfides (4.985 μg/g), ketones (1.254 μg/g), ethers (8.888 μg/g), alkaloids (0.357 μg/g), heterocyclic polymers (2.684 μg/g), cyclomethycaines (1.854 μg/g), polyolefins (0.214 μg/g), furfuryl and furan derivatives (0.987 μg/g), phenols (0.666 μg/g) and diterpenes(1.256 μg/g).

Conclusions: It is noticeable that in absence of oP, the highest level of mP could not improve the volatile compounds, but higher rate of oP even under lower portion of mP recorded higher concentration of the volatile compounds in garlic leaves. Therefore, combined application of organic and mineral P is recommended for better yield and quality of garlic.

Keywords: chlorophyll content, morphology, physiology, organic and mineral phosphorus, volatile compounds

1. Introduction

Assumed to be the second most significant crop producing bulbs, after onions, garlic (*Allium sativum* L.), which is a member of the Lilliaceae family (Tindal 1986, Ledezma and Apitz-Castro 2006), is grown worldwide (Kim et al., 2004, Hamma 2013). In addition to its use as a seasoning in the production of food for humans, it contains extraordinary medical capabilities as well as properties that are particular to the human body's capacity for strength (Etoh and Simon 2002). Garlic production in the world exceeds 22.23 million tons, and among the continents, Asia contributes >80% towards its total production. China leads in garlic production (18.56 million tons) which is ~77% of world garlic production followed by India, Pakistan, South Korea, Egypt, Russia, Myanmar, Ethiopia, the USA, Bangladesh and Ukraine (Khodadadi and Nosrati 2012). A 100 g edible garlic portion contains protein 6.4 g, fat 0.5 g, carbohydrates 33.1 g, calcium 181 g, sodium 17 mg and vitamin C 10. 8 mg (Lorenz and Maynard 1988, Corzo-Martínez et al., 2007). When fried with meat, eggs, and other vegetables, garlic leaves are eaten as a vegetable. In addition to their fresh use, cloves are kept and ground to replace fresh garlic (Amagase 2006). Garlic's sulfur, alliin, and allicin constituents are what give food intended for human consumption its taste. Garlic's alliin content lowers blood sugar and blood pressure, and its special ability to relax vascular smooth muscles prevents hypoxia from increasing under situations of pulmonary pressure (Fallon et al., 1998, Chandrashekara and Venkatesh 2016). Additionally, garlic is a natural remedy for conditions such as diabetes, high blood pressure, cancer, and obesity, and its pungent green tips and bulb are good for flavoring meals (Farooqui et al., 2009).

The soil nutritional balance is essential for successful crop production, and nutritional imbalance may adversely affect crop yields (Frossard et al., 2000). In addition to nitrogen, phosphorus (P) is a nutrient that is necessary for the expansion and development of plants. Additionally, it plays a part in absorbing the energy of the sun and transforming it into molecules that are beneficial to plants (Griffith 2010). The changes of phosphorus and its mobility within the soil-plant system are governed by a confluence of biological, chemical, and physical processes (Frossard et al., 2000, Zaidi et al., 2009). However, overall P-use efficiency of applied P-fertilizers is lower than optimal and only 15-20% of applied P is recovered by the first crop (Vance 2001). Therefore, addition of sufficient P through organic sources is direly needed. Phosphorus is applied in various forms, including DAP, NP, SSP, rock phosphate and phosphor compost etc. (Memon et al., 2012), while P application through farmyard manure (FYM) is one of the most effective source that helps to improve soil organic matter sustainably (Sharif et al., 2000, Singh and Singh 2005). The fertility of the soil may be maintained for an extended period with the use of organic fertilizers. On the other hand, farmers seldom replace the organic matter that is removed from the soil at a rate that is sufficient to offset the loss. Because of this, it is imperative that research be conducted into the potential applications of the many indigenous sources of plant nourishment that are now under development, such as organic and bio fertilizers.

When it comes to the management of soil and nutrients, the primary goals of organic farming are to conserve and maintain soil fertility via the development of biological activity, nitrogen self-sufficiency, and crop variety, and to limit effects on the ecosystem as a whole (Stockdale et al., 2002). There is a wide variety of organic farming methods, each of which takes a unique philosophical and operational approach to the management of soil, plants, and animals (Stockdale et al., 2002). The use of manures resulted in a 12% increase in the harvest, with the manure from chicken coops proving to be the most beneficial (Mahavishnan et al., 2006). In a similar vein, the soils have depleted their supply of accessible phosphorus, and if an appropriate supply of phosphorus is not added to the soil, it will not be possible to achieve the levels of garlic production that are required. Therefore, by using organic P, it is possible to increase the yields of garlic. The application of FYM at a rate corresponding to the needed amount of P may, however, result in a sustained yield boost owing to an improvement in soil organic matter. Inadequate quantities of phosphorus cause a decrease in crop yields in bulbous crops. This is due to the fact that higher quantities of phosphorus are required for the development of healthy bulblets and bulbs, as well as the development of root areas and leaf areas, and as a result, desired bulb yields. On sandy loam soil, the application of phosphorus has demonstrated a high reaction, leading to improved bulb yields in garlic. Due to the fact that phosphorus plays a significant part in root expansion, it is constantly moving through the soil, and its absorption is connected with soil diffusion near the roots and concentration gradients (Robertson and McPharlin 1999). Significant changes in the growth characteristics of garlic were seen when organic manures were used in conjunction with lower application rates of inorganic fertilizer.

The sulfides and thiosulfinates found in Allium plants have antibacterial capabilities against different bacteria and fungi. The volatile chemicals found in Allium plants are discovered by GC-MS and HPLC studies (Yolcu 2011). Disulfides were found to be the main sulfur compounds in leaf extracts containing allicin, which highlighted the possibility of using fresh Allium leaves as a condiment and preservative in the food business. The high culinary and medicinal value of plants in *Allium* genus contain several phytochemicals that signifies its importance in the discovery of new drugs for treatment of cancer (Alizadeh et al., 2013), hepatic disorders, and impotency (SEO et al., 2001). Several organic sulphur compounds in *Allium* species have preventive roles in development of human cancer, cardiovascular diseases and inflammatory diseases (Chandrashekara and Venkatesh 2016). *Allium* plant extract improves antioxidant enzyme activity of diabetic patients (Ledezma and Apitz-Castro 2006), reduces area of renal pathogenic damage induced by adenine and decreases serum level creatinine and blood urea nitrogen(Bede and Zaixiang).

This study investigated the interactive effect of organic and mineral P-sources on morphological and physiological variation in garlic. The variation associated with the volatile compounds due to various P-sources was also documented. It was hypothesized that combined application of organic and mineral P would improve the morphological, physiological, and biochemical attributes of garlic compared with sole application of mineral P.

2. Materials and methods

2.1. Experiment details

Different treatments included T_0 = control, T_1 = 50 kg ha^{-1} mP, T_2 = 70 kg ha^{-1} mP, T_3 = 90 kg ha^{-1} mP, T_4 = 50 kg ha^{-1} mP + oP ≈ 50 kg ha^{-1}, T_5 = 70 kg ha^{-1} mP + oP ≈ 60 kg ha^{-1}, T_6 = 90 kg ha^{-1} mP + oP ≈ 30 kg ha^{-1}, and T_7 = 50 kg ha^{-1} mP + oP ≈ 90 kg ha^{-1}. The whole amount of P was applied at the time of sowing. Single super phosphate was used as a source of mineral P, whereas farmyard manure was used as a source for organic P. The experiment was conducted according to the completely randomized design with four replications.

2.2. Morphological and physiological traits

The measurements of morphological traits were performed by measuring tape (0.01cm); and electronic vernier caliper (0.01mm) was also used for measurement of size wherever necessary. The physiological indices of garlic were determined from leaf samples. Using a spectrophotometer, the chlorophyll content of the leaves was measured. Chlorophyll concentrations were measured in the lab using a dual-wavelength meter of 645 and 663

(Chlorophyll meter model SPAD 502, produced by Minolta Camera Co., Ltd., Japan). At the plant's spike stage, one centimeter of a leaf was removed, 4 ml of acetone was added, and the leaf was then left at room temperature for 24 hours. Using the formula (20.2 A645) + (8.02 A663), this device automatically provided measurements for each sample after storing the sample. Utilizing the dye 2, 6 die-chlorophenol indophenols, vitamin C was measured. Five milliliters of sample were added to a 100 milliliter conical flask along with five milliliters of a Meta phosphoric acid solution (4%) and titrated with 2, 6, and indophenols until a bright pink hue was seen as the final result. Similar to that, a digital pH meter was used to determine pH. By preparing extract of bulb 1:2.5 (bulb extract and water) in a lab, the pH was determined. The pH meter's electrodes were put into the suspension, and measurements that were shown on the device were recorded. The average yield was determined by calculating and weighing the bulbs obtained from each plot and was converted into yield per hectare using the following formula:

$$\text{Yield per hectare} = \frac{\text{Bulb yield per plot}}{\text{Plot size}} \times 10000$$

2.3. Characterization of volatile sulfur-containing compounds

2.3.1. HS-SPME/GC-MS (headspace solid phase micro-extraction combined with gas chromatography-mass spectrometry)

In each of the eight treatments, three randomly harvested garlic plants were submerged in water. Following the removal of the leaves, the plants were carefully shredded apart from one another. Finally, the shredded garlic was crushed in a mortar with 10 ml of deionized water and a small quantity of sea sand to produce a sample of 10 grams for each plant.

2.3.2. HS-SPME analysis

In order to accomplish this goal, univariate analyses (Wang et al., 2020) were carried out. After removing the garlic leaf samples from the liquid N and quickly grinding them to homogenize them, the resulting homogenate (1.0–3.5 g) was placed in a screw-head headspace vial (15 ml) along with a magnetic stirring rotor and ultrapure water (2 ml) to ensure that it was well mixed. After that, a definite amount of NaCl, ranging from 0 to 2.5 grams, was obtained. Ten microliters of an internal standard containing 2-octanol was then added, and the sample vial was sealed using a PTFE silicon stopper. On a metal heating agitation platform afterwards, the headspace container was maintained in equilibrium for a particular amount of time ranging from 5 to 30 minutes at a certain temperature ranging from 30 to 80 degrees Celsius (500 rpm). After that, the removal and adsorption processes were carried out by placing prepared SPME fiber in a headspace bottle for a predetermined amount of time (ranging from ten to sixty minutes) while maintaining constant agitation and heating. After the extraction process was finished, the fiber was desorbed into the GC injection port for a certain amount of time (ranging from one to eleven minutes), and then GC–MS analysis was performed.

2.3.3. GC–MS analysis of volatiles leaf extract

The GC–MS analysis of volatiles leaf extract was used to characterize the volatile sulfur-containing chemicals. For the purpose of separating and identifying the volatile organic compounds, a workstation equipped with an Agilent 7890B Gas Chromatograph, an Agilent 7000D Quadruple Mass Spectrometric Detector (both manufactured by Agilent in the United States), and a standard mass Spectrometry Library (NIST, 2014) was utilized (VOCs). As the stationary phase, a DB-WAX elastic quartz capillary column with dimensions of 30 meters by 0.25 millimeters and 0.25 micrometers was used (Agilent, USA).

Under the following circumstances, the volatile organic compounds (VOCs) of garlic were evaluated using an Agilent 7890B/7000D GC-MS: capillary column, DB-WAX (30 m 0.25 mm, 0.25 m) with He (99.999% purity) as the carrier gas at a flow rate of 1 mL/min and split less mode; initial temperature 40 °C kept for 1 minute, rose to the 80 °C at 8 °C/min, then raised to the 130 °C at 2 °C/min, and lastly elevated to 220 °C at 6 °C/ As soon as the software was activated, the volatile organic compounds were split apart and named using a GC-MS equipped with an automated deconvolution system (AMDIS) and a mass spectrometry library (NIST, 2014). Only those with a matching score of greater than 70 when compared to the Mass Spectrometry Library were found to be relevant for identification. The concentration of VOCs was analyzed by the internal standard method, using the following formula:

$$\text{VOCs}\ (\mu g/kg) = \frac{A1}{A2} \times \frac{M1}{M2} \times 1000$$

Where A1 and A2 are the peak areas of determinant and the internal standard, respectively; M1 and M2 are the amount of the internal standard and sample, respectively.

SPME fiber: 85 µm CAR/PDMS, sample weight: 1.5 g, Na_2SO_4 amount: 0.75 g, extraction temperature: 70 °C, equilibration time: 15 minutes, extraction time: 50 minutes, desorption time: 5 minutes.

2.4. Statistical analysis

The data were assessed for their variation in morphological and quality traits by using one-way analysis of variance (ANOVA). Tukey's post hoc test ($P<0.05$) was used to compare the means where ANOVA denoted significant differences.

3. Results

3.1. Agronomic indices

The treatment effect had a substantial impact on the agronomic indices measured for the garlic crop (P less than 0.05). The combined application of oP and mP resulted in a substantial and beneficial reaction in the height of garlic plants (P less than 0.05). T6 produced the plants that were 67.36 centimeters in height, but other mP-oP combinations produced shorter plants. In the case of seedling emergence, the impact of treatment was likewise significant (P 0.05), with T7 producing earlier germination than the other treatments. Excluding oP had a negative impact on the plant's foliage and caused it to produce a maximum number of leaves (6.83) plant^{-1}. This treatment likewise produced a maximum number of leaves. The T5 treatment resulted in the longest leaf length (42.2 cm), while the other treatments produced shorter leaves (Table 1). The combined action of mP and oP had a profound influence on the morphological and physiological characteristics, as well as the contents of the volatile sulfur containing compounds, of the plants. T7 fertilized garlic plants produced the most bulbs (4382.96 kg ha-1), followed by T5 fertilized plants (4205.43), T6 fertilized plants (4164.10 kg ha-1), T2 fertilized plants (3992.73 kg ha-1), and T4 fertilized plants (3916.30 kg ha-1). T3 had a harvest of 3722.80 kg ha-1, but T1's yield was just 3509.95 kg ha-1.

When comparing the average bulb weight of garlic grown in each mP - oP combination, we found a significant (P0.05) effect of mP and oP interaction. Bulb weights of 42.66 g, 38.90 g, and 37.10 g were all reported in T7 fertilized crops, followed closely by 37.10 g and 37.10 g in T6 and T5 treated plots. T4-fertilized plots showed a little drop in bulb weight (35.16 g). To a similar extent, T7 fertilized crops produced the largest bulbs (4.50 cm in diameter), followed by T6 and T5 (4.40 cm) and T4 (4.40 cm). The largest yield of cloves was found in crops fertilized with T7 (8.50 bulb-1), followed by yields of 8.36, 8.30, 8.20, and 8.20 cloves bulb-1 in T5, T4, and T6 plots, respectively. The best yield of bulbs (4382.96 kg ha-1) was achieved from a crop treated with T7, followed by treatments based on T5, T6, and T4, with yields of 4209.43, 4164.10, 3992.73, and 3916.30 kg ha-1, respectively. In T3-only and T2-only plots, the yield dropped to 3722.80 and 3509.95 kg ha-1, respectively (Table 2).

3.2. Physiological indices

Leaf area was significantly affected ($P<0.05$) by mP–oP integration, and it was higher (83.83 and 83.33 cm) when applied T_7 and T_4, followed by application of mP 70 kg + oP ≈ 60 kg P and mP 90 kg + oP ≈ 30 kg P ha^{-1}; with 82.10 and 75.50 cm leaf area, respectively. Fresh leaf weight was highest (2.70g) when treated with mP 50 kg + oP ≈ 90 kg P ha^{-1}; closely followed by 2.52 g and 2.50 g in mP 90 kg + oP ≈ 30 kg P and mP 70 kg + oP ≈ 60 kg P ha^{-1}, respectively. Similarly, leaf dry weight was higher (0.49 g) in mP 70 kg + oP ≈ 60 kg P ha^{-1}, followed by 0.45, 0.45 and 0.43 g in plots given mP 50 kg + oP ≈ 90 kg P, mP 50 kg + oP ≈ 50 kg P and mP 90 kg + oP ≈ 30 kg P ha^{-1}, respectively. Moreover, highest leaf chlorophyll content (11.9%) was determined in plots given mP 50 kg + oP ≈ 90 kg P ha^{-1}, while 11.60, 11.37 and 10.83% leaf chlorophyll contents were determined in plants given mP 70 kg + oP ≈ 60 kg P, mP 90 kg + oP ≈ 30 kg P ha^{-1} and mP 50 kg + oP ≈ 50 kg P ha^{-1}, respectively (Table 3). There was a linear decrease in leaf chlorophyll (10.39, 10.30 and 9.58%) organic P (oP) was skipped.

A significant variation ($p<0.05$) in VC content was recorded and highest (6.43%) value was determined in plants fertilized with mP 50 kg + oP ≈ 90 kg P ha^{-1}; while 6.16, 6.07 and 5.72% VC was determined in treatments mP 70 kg + oP ≈ 60 kg P, mP 50 kg + oP ≈ 50 kg P and mP 50 kg P ha^{-1}, respectively. The leaf extract pH was highest (6.17) in plants treated with mP 50 kg + oP ≈ 90 kg P ha^{-1}; while it decreased to 6.15, 6.15, 6.14, 6.13 and 6.12 in treatments mP 90 kg + oP ≈ 30 kg P, mP 90 kg P, mP 50 kg ha^{-1}, mP 70 kg + oP ≈ 60 kg P ha^{-1} and mP 50 kg + oP ≈ 50 kg P ha^{-1}, respectively (Table 4).

The characterization of sulfur-containing compounds in plants treated P 50 kg ha^{-1} as SSP+FYM≈90 kg P (T_7) by GC–MS and HPLC analyses detected enormously higher count of constituents in garlic which include Aldehydes(3.254 μg/g), Hydrocarbons (1.245 μg/g), Esters (0.547 μg/g), Acids (1.658 μg/g), Sulfides (4.985 μg/g), Ketones (1.254 μg/g), Ethers (8.888 μg/g), Alkaloids (0.357 μg/g), Heterocyclic polymers (2.684 μg/g), Cyclomethicone (1.854 μg/g), Polyolefins (0.214 μg/g), Furfuryl & furan derivatives (0.987 μg/g), Phenols (0.666 μg/g) and Diterpenes (1.256 μg/g) (Figs. 1-4). The results proved that farmyard manure application improved the soil organic matter; hence the volatile composition increased tremendously.

4. Discussion

The integrated use of organic P (oP) and mineral P (mP) was more effective than their individual application and remarkable improvement in plant height with was observed. The mP 90 kg + oP ≈ 30 kg P ha^{-1} proved to be an effective combination to maximize plant height. These findings are consistent with those of Reddy et al. (2000) and Sardi et al. (2005), who found that integrating FYM with mineral fertilizers led to significant increases in garlic plant height. The mineral and organic P combination relatively delayed seedling emergence, and mP without oP regardless of dose resulted in early germination and more leaves $plant^{-1}$. The combination also resulted in an increase in leaves number suggesting association of improvement with FYM addition. These results are consistent with those of Singh and Singh (2005) and Suthar (2009), who believed that the incorporation of FYM into an NPK fertilizer program resulted in enhanced foliage in garlic. Both of these researchers came to this conclusion after doing research on the subject. According to Chandrashekar et al. (2011), an increase in the combined application of P fertilizers and FYM led to an increase in the number of leaves on garlic plants. Leaves length was significantly higher (42.2 cm) in crop given mP 70 kg + oP ≈ 60 kg P ha^{-1}; this might be associated with improved soil organic matter due to addition of FYM. Akinyele et al. (2012) was of the experience that recommended P in addition to equivalent rate of FYM not only increased the leaves elongation, but the soil organic matter was also improved considerably.

An optimal nutrient balance in the soil is crucial for fruitful crop development, and a deficiency or excess in one area of soil's nutrients may have a negative effect on harvest results (Frossard et al., 2000). Phosphorus (P) is a nutrient that, like nitrogen (N), is important for a plant's growth and development. It also contributes to the process by which the sun's rays are converted into molecules that plants can use (Griffith 2010). A plethora of biological, chemical, and physical mechanisms drive the transformations of phosphorus and its mobility within the soil-plant system (Frossard et al., 2000, Zaidi et al., 2009). Nonetheless, the overall P-use efficiency of P-fertilizers is subpar, and only around 15-20% of applied P is recovered in the first crop (Vance 2001). Therefore, it is crucial to supplement adequate P from organic sources. Farmyard manure (FYM) is one of the most effective sources that helps to sustainably build soil organic matter, among other P sources including DAP, NP, SSP, rock phosphate, and phosphor compost etc. (Memon et al., 2012). (Sharif et al., 2000, Singh and Singh 2005). By using organic fertilizers, soil fertility may be preserved for longer periods of time. But farmers seldom replenish the organic matter taken from the soil at a pace enough to balance the loss. This is why it is crucial to study the uses of the numerous newly developed organic and bio fertilizers and other indigenous sources of plant sustenance.

The bigger leaf area (83.83 cm), leaf fresh weight (2.70 g), leaf chlorophyll concentration (11.9%), and vitamin C concentration (6.43%). a crop treated with mP 50 kg + oP 90 kg P ha-1 was successful. The removal of FYM treatment had a detrimental influence on physiological features, but its reintroduction had a good impact on this trait. The application of phosphorus in mineral form with an equal quantity of FYM resulted in an increase in the leaf area of garlic, and with the addition of FYM, the leaves were broader than when MP was applied alone (Bhandari et al., 2012). Bhandari et al. (2012) discovered that the addition of FYM to the prescribed amount of mineral fertilizers boosted the leaf weight of garlic. Chandrashekar et al. (2011) and Moradi (2015) said that farmyard manures are an effective source of NPK fertilizers that appropriately balance the nutritional requirements of garlic and result in a greener leaf color owing to an increase in chlorophyll content. The results of Abbas et al. (2006) on the vitamin C content of garlic were comparable to those of the current research.

Among yield attributes, maximum bulb diameter (4.50 cm), bulb weight (42.66 g), cloves bulb^{-1} (8.50) and bulb yields (4382.96 kg ha^{-1}) were achieved in crop fertilized with mP 50 kg + oP ≈ 90 kg P ha^{-1}, so the increase in bulb weight under mP – oP combination regardless of nutrient rate. Akinyele et al. (2012) and Adewale et al. (2011) found that using a mix of mineral fertilizers and manures led to greater improvements in bulb size and weight than using mineral fertilizers alone. Several studies, including those by Zakari et al. (2014), Moradi (2015), Nainwal et al. (2015), and Shafeek et al. (2015), found that combining the use of FYM with mineral P increased both bulb size and output. Bulblets per bulb increased when inorganic fertilizers were combined with farmyard manure at P values determined by Shafeek et al. (2015). The combination of mP and oP found to be the most successful in increasing yields, whereas oP was unnecessary and reduced bulb production even when administered at the recommended amount of mP. This shows that the use of FYM improved soil organic matter and hence improved soil fertility causing higher crop yields. This study's findings on bulb yield are consistent with those of many others(Reddy et al., 2000, Sardi et al., 2005, Singh and Singh 2005, Suthar 2009, Adewale et al., 2011, Akinyele et al., 2012, Bhandari et al., 2012, Zakari et al., 2014, Moradi 2015, Nainwal et al., 2015, Shafeek et al., 2015).

The treatment effect on volatiles detected through GC–MS and HPLC analysis showed that P 50 kg ha^{-1} as SSP+FYM≈90 kg P resulted in higher volatile compounds in garlic including aldehydes, hydrocarbons, esters, acids, sulfides, ketones, ethers, alkaloids, heterocyclic polymers, cyclomethicone, polyolefins, furfuryl and furan derivatives, phenols and diterpenes. Our results reflected a linear development in the development of the compounds in garlic leaves and this increase in the compounds was proportional to the quantity of the farmyard manure application. The interaction between the effects of mineral and organic had a major impact not only on the morphological and physiological characteristics of the organism, but also on the contents of the volatile sulfur-containing compounds. The best bulb yields were obtained by garlic plants fertilized with T_7. The response of volatile sulfur-containing compounds in garlic leaves suggested that an enormously larger count of components in garlic leaves were determined in T_7. The total volatile contents of garlic leaves ranged from 29.849 g/g (T_7) to 7.306 g/g on average (control).

Inhibitory effects against several bacteria and fungus may be attributed to the sulfides and thiosulfinates present in Allium plants. Exploratory gas chromatography–mass spectrometry–high-performance liquid chromatography analyses of Allium plants reveal their volatile chemical components (Yolcu 2011). Results showed that disulfides were the primary sulfur compounds in allicin-containing leaf extracts, suggesting that fresh Allium leaves might be used as a condiment and preservative in the food industry. Plants of the Allium genus have several phytochemicals, indicating their potential usefulness in the development of novel medications for the treatment of cancer (Alizadeh et al., 2013), hepatic diseases, and impotence due to their great culinary and medical value (SEO et al., 2001). Allium species include a number of organic sulphur compounds that have been linked to a reduced risk of cancer, cardiovascular disease, and inflammation in humans (Chandrashekara and Venkatesh 2016). Improvements in antioxidant enzyme activity, decreased area of renal pathogenic damage generated by adenine, and lower serum levels of creatinine and blood urea nitrogen have been seen in diabetic patients who took allium plant extract (Ledezma and Apitz-Castro, 2006). (Bede and Zaixiang).

It is noticeable that in absence of farmyard manure, the highest level of mineral P could not improve the volatile compounds, but higher rate of farmyard manure even under lower portion of mineral P recorded higher concentration of the volatile compounds in garlic leaves. P application through FYM is one of the most effective sources that helps to improve soil organic matter sustainably (Sharif et al., 2000). The soil's fertility may be maintained over the long term by the use of organic fertilizers. It has been stated (Singh and Singh, 2005) that farmers often don't replenish the soil with enough organic matter to compensate for the amount that is taken away. In this situation, it's important to investigate the potential for using the increasing number of indigenous sources of plant nourishment, such as organic and bio fertilizers. It's notable that even the greatest concentration of mineral P had no effect on the volatile compounds in garlic leaves without the addition of farmyard manure, whereas a greater rate of farmyard manure resulted in a larger concentration of volatile compounds despite a smaller percentage of mineral P. For optimal growth and flavor, garlic benefits from a combination of organic and mineral P.

5. Conclusions

The morphological and physiological traits as well as contents of the volatile sulfur containing compounds were significantly affected by the interactive effect of mineral and organic. The garlic plants fertilized with T_7 produced highest bulb yields, The response of volatile sulfur containing compounds in garlic leaves indicated that in T_7 enormously higher count of constituents in garlic leaves were determined. The total volatile contents in garlic leaves were in the range of 29.849 μg/g (T_7) and 7.306 μg/g (control). It is noticeable that in absence of farmyard manure, the highest level of mineral P could not improve the volatile compounds, but higher rate of farmyard manure even under lower portion of mineral P recorded higher concentration of the volatile compounds in garlic leaves. Therefore, combined application of organic and mineral P is recommended for better yield and quality of garlic.

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Table 1: Plant height, days to germination, leaves plant^{-1} and leaf length of garlic as affected by organic P (oP) and mineral P (mP) integration

Sr#	Treatments	Plant height (cm)	Days to germination	Leaves number plant^{-1}	Leaf length (cm)
T_0	Control (untreated)	52.36^{f}	18.45^{d}	4.50^{e}	35.86^{c}
T_1	P50 kg ha^{-1}as SSP	52.53^{f}	19.46^{c}	5.13^{d}	38.70^{bc}
T_2	P70 kg ha^{-1}as SSP	56.13^{e}	19.77^{c}	5.40^{cd}	39.13^{ab}
T_3	P90 kg ha^{-1}as SSP	59.26^{d}	20.27^{b}	5.60^{c}	39.03^{b}
T_4	P50 kg ha^{-1} as SSP+FYM≈50kgP	64.95^{b}	20.34^{b}	6.83^{a}	41.40^{ab}
T_5	P 70 kg ha^{-1} as SSP+FYM≈60kgP	62.56^{c}	50.39^{a}	6.50^{b}	42.20^{a}
T_6	P 90 kg ha^{-1} as SSP+FYM≈30kgP	67.36^{a}	21.67^{a}	6.30^{b}	40.26^{ab}
T_7	P 50 kg ha^{-1} as SSP+FYM≈90kgP	66.87^{ab}	21.44^{a}	6.43^{b}	39.90^{ab}
	S.E.±	*0.9788*	*0.1612*	*0.1531*	*1.4547*
	LSD 0.05	*2.0993*	*0.3458*	*0.3285*	*3.1199*

Table 2: Weight of bulbs, bulb diameter, cloves bulb^{-1} and bulb yield ha^{-1} of garlic as affected by organic P (oP) and mineral P (mP) integration

Sr#	**Treatments**	**Wt of bulbs plant^{-1}**	**Bulb diameter (cm)**	**No. of Cloves bulb^{-1}**	**Bulb yield (kg ha^{-1})**
T_0	Control (untreated)	23.06^{e}	2.93^{c}	6.20^{d}	2506.90^{f}
T_1	P50 kg ha^{-1}as SSP	26.86^{de}	3.70^{b}	7.80^{b}	3509.93^{e}
T_2	P70 kg ha^{-1}as SSP	28.96^{de}	3.80^{b}	8.20^{a}	3992.73^{bcd}
T_3	P90 kg ha^{-1}as SSP	30.10^{cd}	3.86^{b}	8.30^{a}	3722.80^{de}
T_4	P50 kg ha^{-1} as SSP+FYM≈50kgP	35.16^{bc}	4.40^{a}	7.26^{c}	3916.30^{cd}
T_5	P 70 kg ha^{-1} as SSP+FYM≈60kgP	37.10^{b}	4.40^{a}	8.36^{a}	4205.43^{ab}
T_6	P 90 kg ha^{-1} as SSP+FYM≈30kgP	38.90^{b}	4.40^{a}	8.20^{a}	4164.10^{abc}
T_7	P 50 kg ha^{-1} as SSP+FYM≈90kgP	42.66^{a}	4.50^{a}	8.50^{a}	4382.96^{a}
	S.E.±	*2.7701*	*0.1529*	*0.1512*	*130.42*
	LSD 0.05	*5.9413*	*0.3279*	*0.3243*	*279.72*

Table 3: Leaf area, fresh/dry leaf weight and chlorophyll content of garlic as affected by organic P (oP) and mineral P (mP) integration

Sr#	**Treatments**	**Leaf area (cm/plant)**	**Fresh leaf weight (g)**	**Dry leaf weight (g)**
T_0	Control (untreated)	50.21^{e}	1.32^{d}	0.23^{d}
T_1	P50 kg ha^{-1}as SSP	53.88^{e}	1.59^{cd}	0.43^{c}
T_2	P70 kg ha^{-1}as SSP	61.97^{d}	1.65^{c}	0.33^{c}
T_3	P90 kg ha^{-1}as SSP	70.38^{c}	1.86^{c}	0.35^{c}
T_4	P50 kg ha^{-1} as SSP+FYM≈50kgP	83.33^{a}	2.22^{b}	0.45^{b}
T_5	P 70 kg ha^{-1} as SSP+FYM≈60kgP	82.10^{ab}	2.50^{ab}	0.49^{a}
T_6	P 90 kg ha^{-1} as SSP+FYM≈30kgP	75.50^{bc}	2.52^{a}	0.43^{b}
T_7	P 50 kg ha^{-1} as SSP+FYM≈90kgP	83.83^{a}	2.70^{a}	0.45^{b}
	S.E.±	*3.3608*	*0.1356*	*0.0194*
	LSD 0.05	*7.2081*	*0.2909*	*0.0416*

Table 4: Chlorophyll content, extract pH and vitamin C in garlic as affected by organic P (oP) and mineral P (mP) integration

Sr#	Treatments	Chlorophyll content (%)	Extract pH	Vit-C content (%)
T_0	Control (untreated)	6.29^{d}	5.16^{b}	3.75^{c}
T_1	P50 kg ha^{-1}as SSP	9.58^{c}	6.14^{a}	5.72^{ab}
T_2	P70 kg ha^{-1}as SSP	10.30^{bc}	6.07^{a}	5.10^{abc}
T_3	P90 kg ha^{-1}as SSP	10.39^{abc}	6.15^{a}	4.56^{bc}
T_4	P50 kg ha^{-1} as SSP+FYM≈50kgP	10.83^{abc}	6.12^{a}	6.07^{ab}
T_5	P 70 kg ha^{-1} as SSP+FYM≈60kgP	11.60^{ab}	6.13^{a}	6.16^{a}
T_6	P 90 kg ha^{-1} as SSP+FYM≈30kgP	11.37^{ab}	6.15^{a}	5.36^{ab}
T_7	P 50 kg ha^{-1} as SSP+FYM≈90kgP	11.90^{a}	6.17^{a}	6.43^{a}
	S.E.±	*0.7171*	*0.0800*	*0.7185*
	LSD 0.05	*1.5381*	*0.1717*	*1.5410*

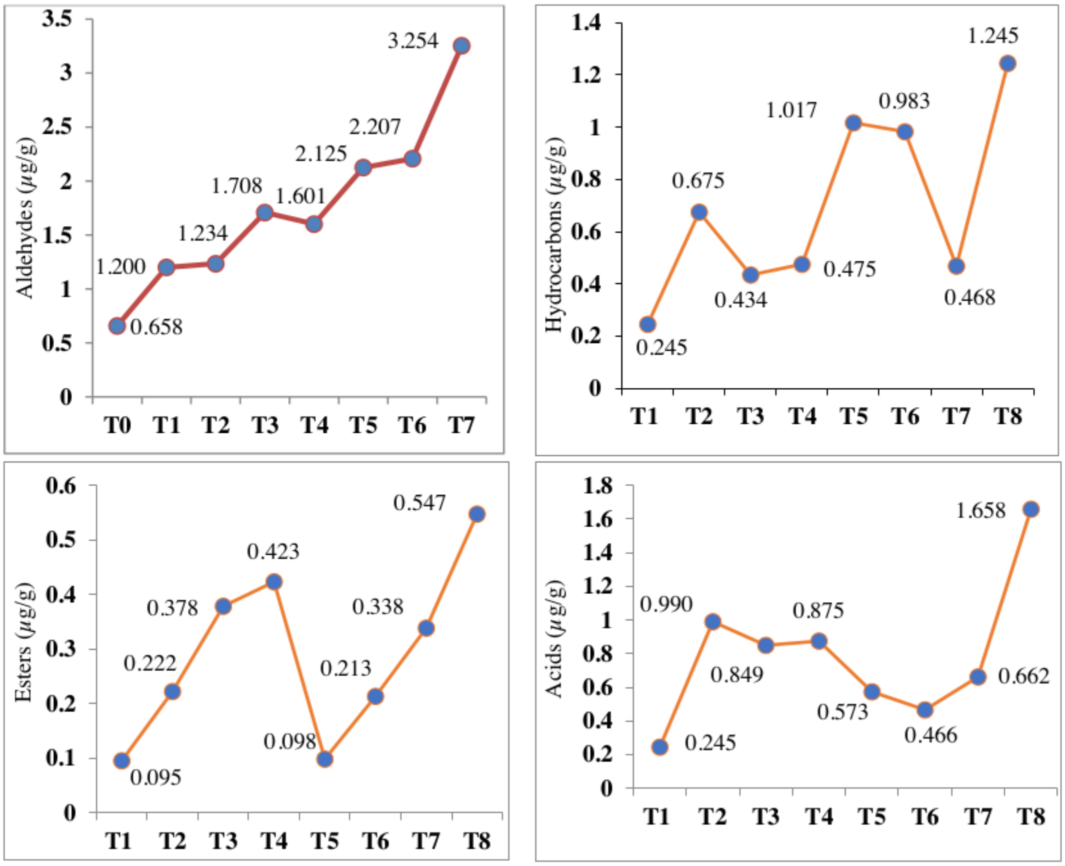

Figure 1. The impact of different forms of phosphorus on aldehydes, hydrocarbons, esters and acids in garlic leaves

Here, T_0 = control, T_1 = 50 kg ha^{-1} mP, T_2 = 70 kg ha^{-1} mP, T_3 = 90 kg ha^{-1} mP, T_4 = 50 kg ha^{-1} mP + oP ≈ 50 kg ha^{-1}, T_5 = 70 kg ha^{-1} mP + oP ≈ 60 kg ha^{-1}, T_6 = 90 kg ha^{-1} mP + oP ≈ 30 kg ha^{-1}, and T_7 = 50 kg ha^{-1} mP + oP ≈ 90 kg ha^{-1}.

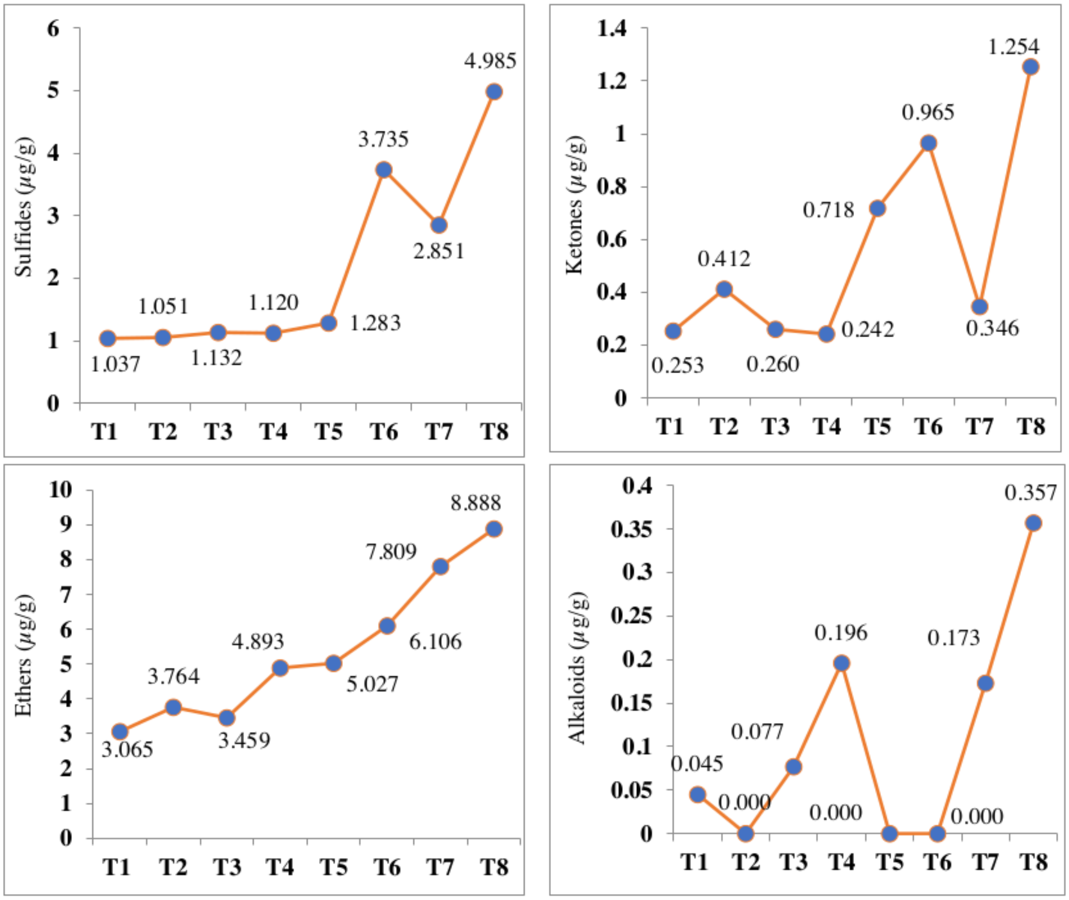

Figure 2: The impact of different forms of phosphorus on sulfides, ketones, ethers, and alkaloids in garlic leaves

Here, T_0 = control, T_1 = 50 kg ha^{-1} mP, T_2 = 70 kg ha^{-1} mP, T_3 = 90 kg ha^{-1} mP, T_4 = 50 kg ha^{-1} mP + oP ≈ 50 kg ha^{-1}, T_5 = 70 kg ha^{-1} mP + oP ≈ 60 kg ha^{-1}, T_6 = 90 kg ha^{-1} mP + oP ≈ 30 kg ha^{-1}, and T_7 = 50 kg ha^{-1} mP + oP ≈ 90 kg ha^{-1}.

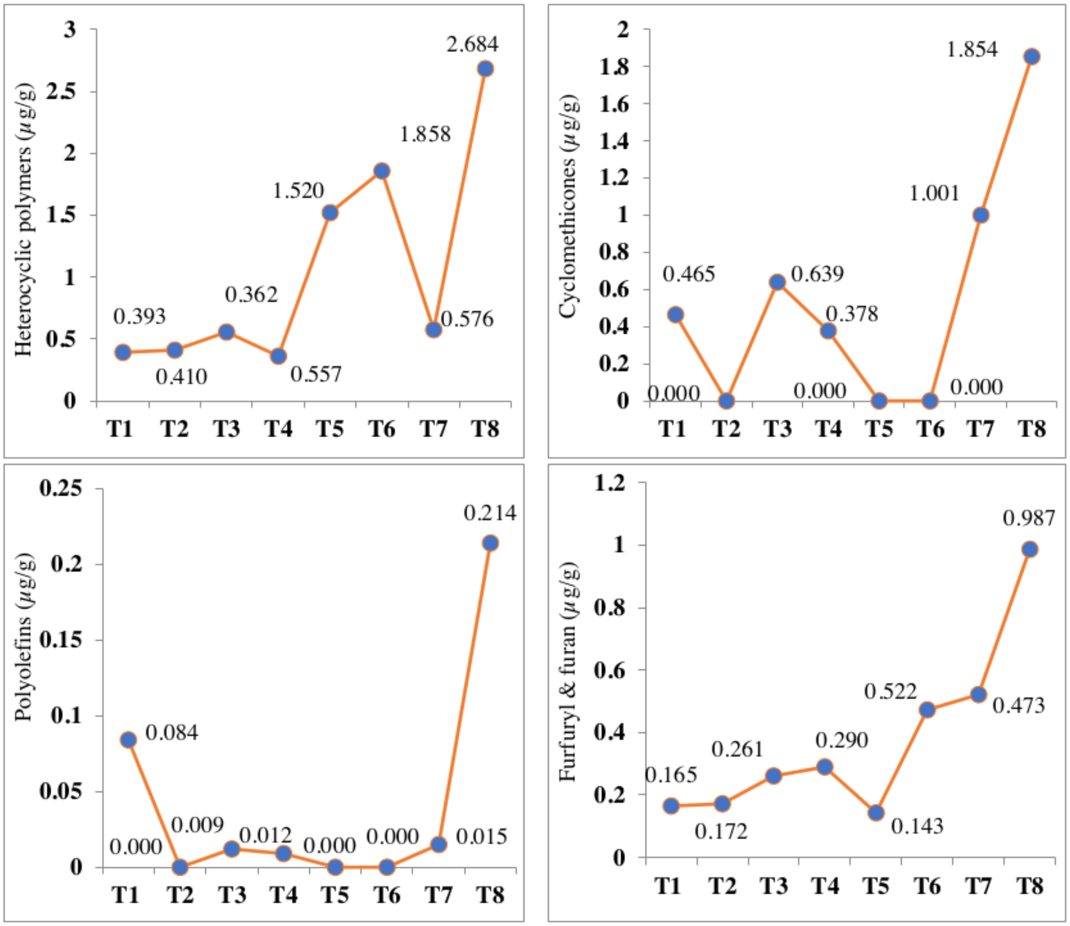

Figure 3: The impact of different forms of phosphorus on heterocyclic polymers, cyclomethicones, polyolefins, and furfuryl and furan in garlic

Here, T_0 = control, T_1 = 50 kg ha^{-1} mP, T_2 = 70 kg ha^{-1} mP, T_3 = 90 kg ha^{-1} mP, T_4 = 50 kg ha^{-1} mP + oP ≈ 50 kg ha^{-1}, T_5 = 70 kg ha^{-1} mP + oP ≈ 60 kg ha^{-1}, T_6 = 90 kg ha^{-1} mP + oP ≈ 30 kg ha^{-1}, and T_7 = 50 kg ha^{-1} mP + oP ≈ 90 kg ha^{-1}.

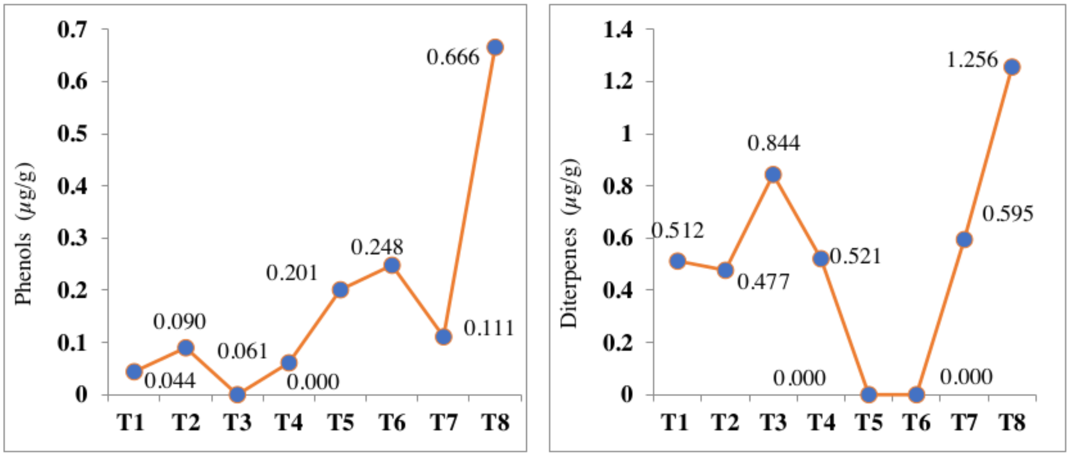

Figure 4: The impact of different forms of phosphorus on phenols and diterpenes in garlic

Here, T_0 = control, T_1 = 50 kg ha^{-1} mP, T_2 = 70 kg ha^{-1} mP, T_3 = 90 kg ha^{-1} mP, T_4 = 50 kg ha^{-1} mP + oP ≈ 50 kg ha^{-1}, T_5 = 70 kg ha^{-1} mP + oP ≈ 60 kg ha^{-1}, T_6 = 90 kg ha^{-1} mP + oP ≈ 30 kg ha^{-1}, and T_7 = 50 kg ha^{-1} mP + oP ≈ 90 kg ha^{-1}.

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