



Integrated management of soil applied organic and inorganic amendments to improve growth and flower quality of chamomile

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Abstract

Depression is one of the alarming psychiatric conditions all over the world which is linked with restlessness. Chamomile has antidepressant properties. Organic manures, apart from improving biological and physical properties of soil and effectiveness of chemical fertilizers also increase the secondary metabolites production as well as antioxidants levels. A trial "Integrated management of soil applied organic and inorganic amendments to improve the growth and flower quality of chamomile" was conducted at Fruit and Vegetable Nursery Harama, the Department of Agriculture Muzaffarabad in two consecutive years during 2016-2018. The trial was designed as RCB with factorial combinations. Recommended dose of commercial fertilizer: NPK at 100:50:50 kg ha⁻¹ was combined with organic fertilizer FYM at 20 t ha⁻¹ and humic acid at 5 kg ha⁻¹ in different doses. Results showed that a hundred flower fresh weight (4.19 g), fresh root weight (8.12 g), root length (7.86 cm), essential oil (0.17 %), fresh flower yield (1109.2 kg ha⁻¹) and dry flower yield (502.21 kg ha⁻¹) were received in plots receiving no treatment (control). The combination NPK at 100:50:50 + FYM at 20 t ha⁻¹ + HA at 5 kg ha⁻¹ showed maximum results for above mentioned attributes i.e. 14.9 g, 19.6 g, 18.6 cm, 0.38%, 2251.6 kg ha⁻¹ and 1054.7 kg ha⁻¹, respectively. Survival percentage was not significantly affected by treatments. Screening of phytochemicals and antioxidant activities showed maximum value for total phenolic content (8 mg g⁻¹ DW), total flavonoid content (554.4 µg mg⁻¹ DW) and antioxidant activity (76.4%) in plants receiving NPK at 50:25:25 + FYM at 20 t + HA at 5 kg ha⁻¹ closely pursued by 7.82 mg g⁻¹ DW, 542 µg mg⁻¹ DW and 70.5% in plants treated with NPK at 50:25:25 + FYM at 10 t + HA at 2.5 kg ha⁻¹, respectively. It is recommended that plants should be fertilized with NPK at 100:50:50 + FYM at 20 t ha⁻¹ + HA at 5 kg ha⁻¹ for maximum growth and yield and for higher concentration of secondary metabolites and antioxidant activities plants should be grown in soil supplied with NPK at 50:25:25 + FYM at 10 t + HA at 2.5 kg ha⁻¹.

Keywords: Chamomile, humic acid, farmyard manure, commercial, yield, essential oil

Introduction

One of the primeval and well-recognized curative herbs *Matricaria chamomilla* is the member of Asteraceae family (Srivastava *et al.*, 2010). It is used as a medicinal plant worldwide (Shams *et al.*, 2012). Essential oil (azulenes, α -bisabolol and its oxides) and phenols (mainly the flavonoids apigenin, quercetin, patuletin, luteolin and their glucosides) present in flower are responsible for biological activity of chamomile (Hadaruga *et al.*, 2009). Owing to its superfluous conventional remedial assets and significance of essential oil, it could be commercialized. Commercialized yield of flowers and volatile oil from chamomile is an innovative step in the agrarian framework. Scarce statistic of data is available on cultivation, proper nutrition, essential oil

extraction and phytochemical analysis of chamomile in Pakistan and especially in Azad Kashmir.

Organic stuff in soil enhanced nutrient uptake (Renato, 2003), porosity, aeration, water holding capacity and infiltration ultimately enhancing crop yield (Rani and Nishana, 2012). Organic produce is healthier than conventionally grown produce due to higher levels of micronutrients (Hunter, 2011). It has been recurrent to use FYM for improving the biological, chemical and physical characteristics of the soil. The influence of FYM on the soil characteristic results in increase in yield (Kuepper, 2003). Humic acid (HA) is not harmful to plant and environment because it is stable with nature (Haghighi *et al.*, 2011). It is dynamic in mineral nutrients transformation into available form (Akinci *et al.*, 2009). Being an active organic

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constituent, humus has an imperative role in the conditioning of soil and crop growing (Shafi *et al.*, 2020). Biologically, it increases the microbial growth, physically it increases water retention and improves soil structure whereas chemically it serves as adsorbent and retainer complex for nutrients (Shafi *et al.*, 2020). Fertility of soil and availability of nutrients like phosphorus can be increased through humic acid application with chemical fertilizers (Shafi and Sharif, 2019).

In case of chamomile few nutritional studies have been conducted under normal soil conditions by earlier workers (Kumar *et al.*, 2016). For improved plant growth, nutrition is one of the most important factors. Keeping in view its economic, industrial medicinal importance, world demands, and its tolerance potential to variable soil conditions, the current study was planned to check the adaptability of chamomile under agro-climatic conditions of Azad Jammu & Kashmir and to optimize the levels of inorganic and organic (farmyard manure and humic acid) sources for maximum growth and quality flower production of chamomile.

Materials and Methods

Azad Kashmir (AK) valley is situated between 73°28'20" East longitude and 34°21'30" North latitude. The capital of Azad Kashmir is Muzaffarabad. Integrated management of soil applied organic and inorganic amendments to improve the growth and flower quality of chamomile was designed for two years. There were three replications and each replication had sixteen treatments. Suggested doses of commercial fertilizer: NPK at 100: 50: 50 kg ha⁻¹ were combined with organic fertilizer FYM at 20 t ha⁻¹ (Singh *et al.*, 2011) and HA at 5 kg ha⁻¹ (Sharif *et al.*, 2006) in different combinations. Factorial combinations of two NPK levels, three organic amendments and their levels along with four treatments were carried out in RCB design.

The treatment combinations were as follow:

T1=without fertilizers (control)

T2=NPK at 100:50:50 kg ha⁻¹

T3=FYM at 20 t ha⁻¹

T4= HA at 5 kg ha⁻¹

T5= NPK at 50:25:25 kg ha⁻¹ + FYM at 10 t ha⁻¹

T6=NPK at 50:25:25 + FYM at 10 t ha⁻¹ + HA at 5 kg ha⁻¹

T7=NPK at 50:25:25 + FYM at 10 t ha⁻¹+ HA at 2.5 kg ha⁻¹

T8=NPK at 100:50:50 kg ha⁻¹+ FYM at 10 t ha⁻¹

T9=NPK at 100:50:50 kg ha⁻¹ + HA at 2.5 kg ha⁻¹

T10=NPK at 100:50:50 kg ha⁻¹+ FYM at 10 t ha⁻¹+ HA at 2.5 kg

T11=NPK at 50:25:25 + FYM at 20 t ha⁻¹

T12=NPK at 50:25:25 + HA at 5 kg ha⁻¹

T13=NPK at 50:25:25 + FYM at 20 t ha⁻¹ + HA at 5 kg ha⁻¹

T14=NPK at 100:50:50 kg ha⁻¹ + FYM at 20 t ha⁻¹

T15=NPK at 100:50:50 kg ha⁻¹ + HA at 5 kg ha⁻¹

T16=NPK at 100:50:50 kg ha⁻¹+ FYM at 20 t ha⁻¹ + HA at 5 kg ha⁻¹

Table 1: Characterization of experimental soil

Parameter	Value
Saturation (%)	48
pH	7.75
Nitrogen (%)	0.11
Phosphorous (mg kg ⁻¹)	3
Potassium (mg kg ⁻¹)	110
Organic matter (%)	0.45
Texture	Clay loam
Depth (cm)	0-15

Table 2: Characteristics of farmyard manure

Parameter	2016-2017	2017-2018
Total organic C (g kg ⁻¹)	476.3	482.4
Nitrogen (%)	0.66	0.72
Phosphorous (%)	0.18	0.17
Potassium (%)	0.71	0.68
pH	7.56	7.49

Characterization of soil and farmyard manure

Prior to use, samples of soil and FYM were tested for organic matter (OM), pH, nitrogen (N), phosphorous (P) and potassium (K). A composite was made by mixing together the samples of soil collected indiscriminately from various sites of the field (15 cm deep) and were carried to Soil and Environmental Science laboratory for the characterization. From local farmhouse, FYM (farmyard manure) was collected and was carried to Lab. for analysis and data are depicted in (table 1 and 2).

Preparation of nursery plants

Pots were used for seed sowing; pots were filled with media and enclosed in thin polyethylene sheet. Plots were designed accordingly and after six to seven weeks seedlings were uprooted to endure a slight shock as possible in outdoor conditions and then transplanted in plots.

Soil preparation

Soil was tilled thoroughly and then leveled through rotavator. The proposed doses of organic fertilizer were incorporated before transplantation of seedlings. Then seedlings were planted 30 cm apart in rows whereas 40 cm was kept between rows and irrigation was done immediately. During the whole crop season plants were



irrigated as per plant requirement. Manual weeding was done in subplots.

Parameters studied

The data were reported on 100 flower fresh weight (g), fresh root weight (g), root length (cm), essential oil (%), fresh and dry yield (kg ha^{-1}), survival (%), total phenolic contents (mg g^{-1} DW), total flavonoid content ($\mu\text{g mg}^{-1}$ DW) and antioxidant activities (%) with standard methods.

Collection of flowers

Fresh flowers were harvested from experimental site and were carried to the Lab. of AJ& K University for extraction of oil.

Extraction of essential oil

Procedure

One of the operational and dynamic techniques for maximum extraction is the hydro-distillation technique by Clevenger hydro-distillation (Ansarifar *et al.*, 2013). As the flowers were flimsy so there was a chance to lose essential oil components so temperature was sustained at low range. The essential oil was collected in Eppendorf tubes and stored in the refrigerator and essential oil (%) was calculated by succeeding formula.

$$\text{Essential oil percentage} = \frac{\text{Weight of oil extracted}}{\text{Total weight of fresh flowers taken}} \times 100$$

Preparation of extract

Flowers were shade dried for 10 days at room temperature then these dried flowers were ground into fine powder with the help of electrical grinder and were stored at 4 °C for further studies. The 100 g dried powder was macerated in 500 mL of methanol and kept at room temperature (25 ± 2 °C) for 10 days and occasional shaking was done. After maceration period, the solvent extracted material was filtered through Whatman No. 1 filter paper. The methanol filtrate was evaporated to dryness using rotary vacuum evaporator (Buchi, Switzerland) under reduced pressure and temperature at 40-45°C to obtain a greenish residue. The dried extracts were kept in an air tight glass jar and stored at 4°C in a refrigerator until used for phytochemical screening and antioxidant activity.

Total phenolic content (TPC)

The total phenolic content was determined by spectrophotometer according to the Folin-Ciocalteu colorimetric method described in Lavanya *et al.* (2016). The 200 μL plant extract was taken in the screw cap test tubes, and 1 mL of Folin-Ciocalteu reagent (1:1 with

water) and 1 mL of sodium carbonate (7.5%) was added. The tubes were vortexed and after 2 h incubation, the absorbance was recorded at 726 nm using a spectrophotometer. The total phenolic content was expressed as gallic acid equivalents (GAE) in mg/g of plant extract.

Total flavonoid content (TFC)

The total flavonoid content was determined by aluminium chloride colourimetric method mentioned in Lavanya *et al.* (2016). The 0.1 mg of plant extract was dissolved in 1 mL of methanol and mixed with 0.1 mL of 10% aluminium chloride hexahydrate, 0.1 mL of 1 M potassium acetate and 2.8 mL of deionized water. The absorbance was taken at 415 nm in spectrophotometer after 40 min of incubation. Quercetin was used as a standard (the concentration range: 0.005 to 0.1 mg mL^{-1}) and the TFC in the crude extracts was determined.

Antioxidant activity (%)

Antioxidant activities were determined through assay 1, 1-diphenyl-2-picryl-hydrazyl (DPPH). The DPPH free radical test was conducted using the method of (Ghasemzadeh *et al.*, 2010). The initial absorbance of DPPH in methanol was measured at 515 nm until the absorbance remained constant. Extracts (40 μL) was added to alcohol solutions of DPPH (3 mL, 0.1 mM). The samples were first kept in the dark place at room temperature and after 30 min the absorbance was measured using a spectrophotometer (U-2001, Hitachi Instruments, Tokyo, Japan) at 515 nm. Finally, the radical quenching activity was calculated as percentage of DPPH discolouration using the following equation:

$$\text{DPPH Scavenging activity (\%)} = \frac{[\text{OD control} - \text{OD sample}]}{\text{OD control}} \times 100$$

Statistical analysis

The collected data of various attributes were examined by ANOVA (analysis of variance) technique to check the difference in treatments and their collaborations. Significantly different means were equated by using LSD (Least Significant Difference) test. For calculating the ANOVA and LSD test statistical computer software was assessed through Statistix 8.1 (Jan *et al.*, 2009).

Results

Current findings on vegetative, yield attributes, secondary metabolites production and anti-oxidant activities of chamomile are given in tables 3, 4 & 5. The interaction between NPK \times OA (organic amendments) and treatment \times year were also observed and are shown in figures (1-7).



Table 3: Hundred flower fresh weight (g), fresh root weight (g) and root length (cm) of chamomile as affected by integration of inorganic fertilizer with farmyard manure and humic acid

Treatment	Hundred flower fresh weight (g)	Fresh root weight (g)	Root length (cm)
Control	4.19 p	8.12 h	7.86 k
NPK	10.0 j	11.1 e	11.0 gh
FYM	7.6 n	9.2 gh	8.5 jk
HA	7.0 o	8.9 gh	8.3 jk
1/2 NPK+1/2 FYM	9.3 k	10.4 ef	10.4 hi
1/2 NPK + 1/2 HA	8.2 m	9.4 fg	8.9 jk
1/2 NPK + 1/2 FYM+1/2 HA	10.5 i	12.5 d	12.3 fg
Full NPK + 1/2 FYM	12.0 f	14.9 c	13.7 ef
Full NPK + 1/2 HA	11.0 h	12.8 d	12.8 f
Full NPK + 1/2 FYM + 1/2 HA	14.6 b	19.5 a	17.6 ab
1/2 NPK + full FYM	11.5 g	14.0 c	13.3 ef
1/2 NPK + full HA	8.6 l	10.0e fg	9.4 ij
1/2 NPK + full FYM + full HA	12.6 e	16.8 b	14.5 de
Full NPK + full FYM	13.9 c	18.6 a	16.8 bc
Full NPK + full HA	13.2 d	17.3 b	15.8 cd
Full NPK + full FYM + full HA	14.9 a	19.6 a	18.6 a
LSD at α 0.05	0.05	1.22	1.46
Interaction			
NPK \times OA	*	NS	NS
T \times Y	*	NS	NS

Considerably dissimilar means were symbolized by different alphabets. 5% significance level is shown by * whereas NS means nonsignificant. NPK \times OA (organic amendments) interaction between NPK levels and organic amendments whereas T \times Y is interaction between treatment and years.

Table 4: Essential oil (%), fresh flower yield (kg ha⁻¹), dry flower yield (kg ha⁻¹) and survival percentage (%) of chamomile as affected by integration of inorganic fertilizers with farmyard manure and humic acid

Treatment	Essential oil Content (%)	Fresh flower yield (kg ha ⁻¹)	Dry flower yield (kg ha ⁻¹)	Survival (%)
Control	0.17 l	1109.2 k	502.21 m	94.17
NPK	0.26 gh	1688.4 f	622.8 h	92.5
FYM	0.23 jk	1266.9 i	511.1 k	95.8
HA	0.22 k	1162.1 j	505.7 l	95.8
1/2 NPK+1/2 FYM	0.258 hi	1604.3 g	591.4 hi	98.3
1/2 NPK + 1/2 HA	0.24 ij	1525.5 h	549.7 j	96.7
1/2 NPK + 1/2 FYM+1/2 HA	0.265 fg	1753.9 ef	680.9 g	99.2
Full NPK + 1/2 FYM	0.276 cde	1921.4 d	776.4 e	99.2
Full NPK + 1/2 HA	0.27 ef	1776.6 e	698.5 fg	98.3
Full NPK + 1/2 FYM + 1/2 HA	0.37 ab	2191.5 ab	995.1 b	95.0
1/2 NPK + full FYM	0.271 def	1810.6 e	727.9 f	96.7
1/2 NPK + full HA	0.25 hij	1538.6 gh	561.9 ij	93.3
1/2 NPK + full FYM + full HA	0.28 cde	1950.2 d	800.4 e	96.7
full NPK + full FYM	0.32 abc	2140.9 b	917.5 c	98.3
Full NPK + full HA	0.29 bcd	2058.2 c	853.2 d	98.3
Full NPK + full FYM + full HA	0.38 a	2251.6 a	1054.7 a	99.2
LSD at α 0.05	0.03	71.61	33.13	Ns
Interaction				
NPK \times OA	NS	NS	*	
T \times Y	NS	NS	*	

Considerably dissimilar means were symbolized by different alphabets. 5% significance level is shown by * whereas NS means nonsignificant. NPK \times OA (organic amendments) interaction between NPK levels and organic amendments whereas T \times Y is interaction between treatment and years.



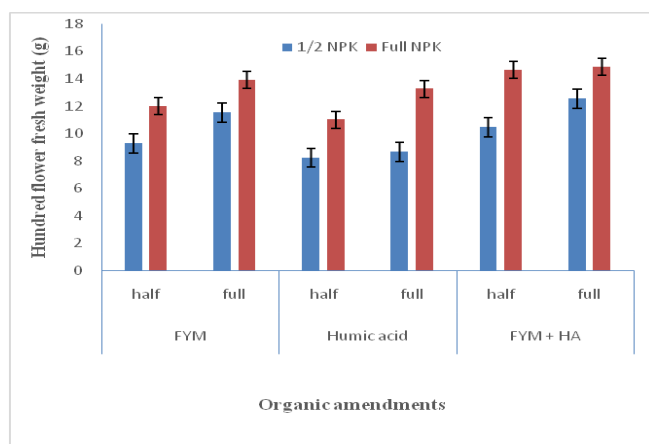


Figure 1: Hundred flower fresh weight (g) of chamomile as affected by integration of inorganic fertilizer with farmyard manure and humic acid

Graph showed interactive effect of organic fertilizers and NPK levels. Full NPK = 100: 50: 50 kg ha⁻¹, half NPK = 50: 25:25, full FYM = 20 t ha⁻¹, half FYM = 10 t ha⁻¹, full HA = 5 kg ha⁻¹ and half HA = 2.5 kg ha⁻¹.

Data regarding hundred flower fresh weight (g), fresh root weight (g) and root length (cm) are depicted in table 3. The analysis of data revealed that growth characters of chamomile were significantly ($p \leq 0.05$) affected by the integrated nutrient management. The interaction between NPK \times OA (organic amendments) and treatment \times year for hundred flowers fresh weight (g) was significantly affected & shown in figures 1 & 2.

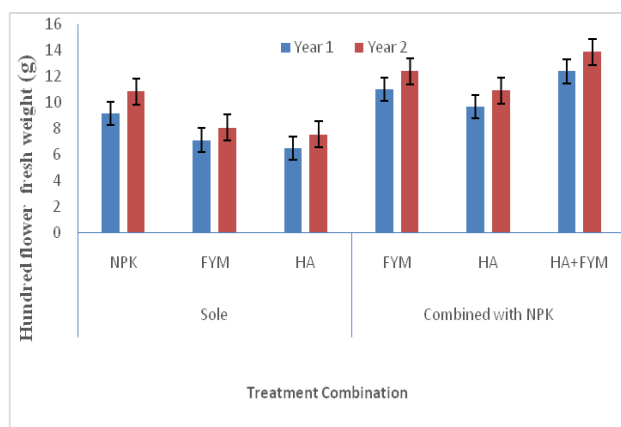


Figure 2: Hundred flower fresh weight (g) of chamomile as affected by year

Year 1= 2016-2017, Year 2= 2017-2018. Interactive effect between hundred flower fresh weight (g) and year (source of variation).

The results illustrated that a minimum hundred flower fresh weight (4.19 g), fresh root weight (8.12 g) and root length (7.86 cm) were noticed in control. An increase was documented in above mentioned attributes in plants treated with NPK at 100:50:50 i.e. 10 g, 11.1 g and 11 cm,

respectively. This revealed the participation of NPK in growing attributes in the current study.

The integration of NPK at 100:50:50 with HA at 5 kg ha⁻¹ and then with FYM at 20 t ha⁻¹ further enhanced these parameters as equated to unfertilized plants and alone NPK i.e. hundred flower fresh weight (13.2 to 13.9 g), fresh root weight (17.3 to 18.6 g) and root length (15.8 to 16.8 cm), respectively. However, maximum results were noticed in plants receiving combined doses of NPK at 100:50:50 + FYM at 20 t + HA at 5 kg ha⁻¹ i.e., hundred flower fresh weight (14.9 g), fresh root weight (19.6 g) and root length (18.6 cm).

Yield and adaptability parameters

Yield of essential oil (%), fresh and dry flower (kg ha⁻¹) were recorded whereas the adaptability was also checked by observing survival percentage. Response in yield parameters to organic amendments and interaction were significantly affected whereas survival percentage was unaffected by organic amendments. Data regarding the yield & adaptability are shown in table 4 and the interaction between NPK \times OA (organic amendments) and treatment \times year for dry flower yield (kg ha⁻¹) are shown in figures 3 & 4.

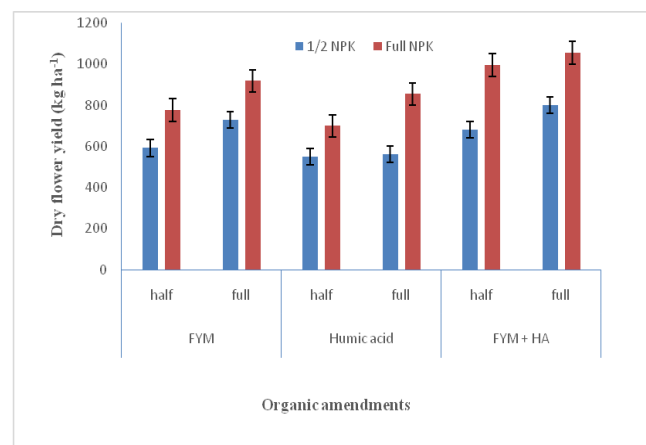


Figure 3: Dry flower yield kg ha⁻¹ of chamomile as affected by integration of inorganic fertilizer with farmyard manure and humic acid

Essential oil (%)

Application of NPK whether applied alone or in conjunction with HA and FYM significantly ($p \leq 0.05$) improved essential oil (%) of chamomile. Concerning about means an improved pattern was observed in essential oil i.e. (0.171%) in the control then it was (0.26%) at full NPK levels (100:50:50). The efficiency of NPK at 100:50:50 was improved with combining with HA at 5 kg ha⁻¹ + FYM at 20 t ha⁻¹ which raised the essential oil content (%) as linked to unfertilized plants i.e. 0.29% and 0.32%, correspondingly.



Similarly, increment in essential oil (0.38%) was observed in plots supplied with NPK at 100:50:50 + FYM at 20 t ha⁻¹ + HA at 5 kg ha⁻¹.

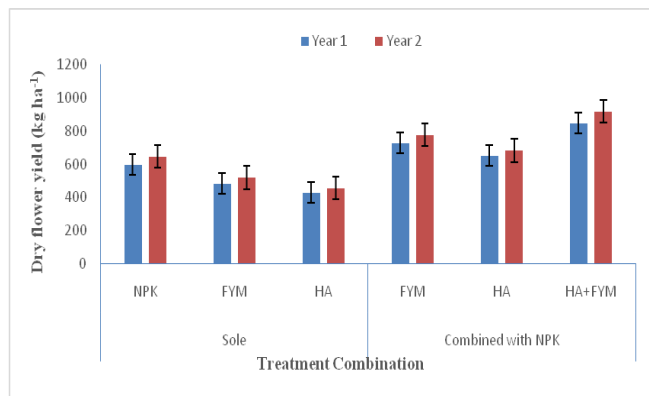


Figure 4: Dry flower yield (kg ha⁻¹) of chamomile as affected by year

Interactive effect between hundred flowers fresh weight (g) and year (source of variation). Year 1= 2016-2017, Year 2= 2017-2018.

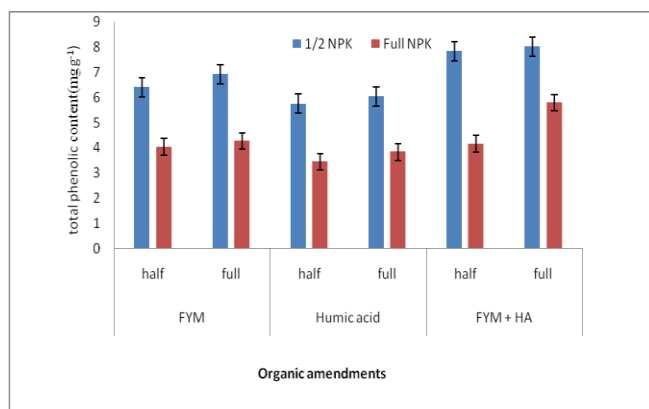


Figure 5: Total phenolic content (mg g⁻¹) of chamomile as affected by integration of inorganic fertilizer with farmyard manure and humic acid

Fresh and dry flower yield (kg ha⁻¹)

Concerning means of fresh flower yield (1688.4 kg ha⁻¹) and dry flower yield (622.8 kg ha⁻¹) were recorded with NPK at 100:50:50 whereas minimum results (1109.2 kg ha⁻¹) and 502.21 kg ha⁻¹ were observed in control treatment. The combination of NPK at 100:50:50 with HA at 5 kg ha⁻¹ and then FYM at 20 t ha⁻¹ improved these parameters i.e. fresh flower yield (2058.2 to 2140.9 kg ha⁻¹) and dry flower yield (853.2 kg ha⁻¹ to 917.5 kg ha⁻¹). Likewise, superior results in above described parameters were recorded in treatment receiving NPK at 100:50:50 + FYM at 20 t ha⁻¹ + HA at 5 kg ha⁻¹ i.e. fresh flower yield (2251.6 kg ha⁻¹) and dry flower yield (1054.7 kg ha⁻¹).

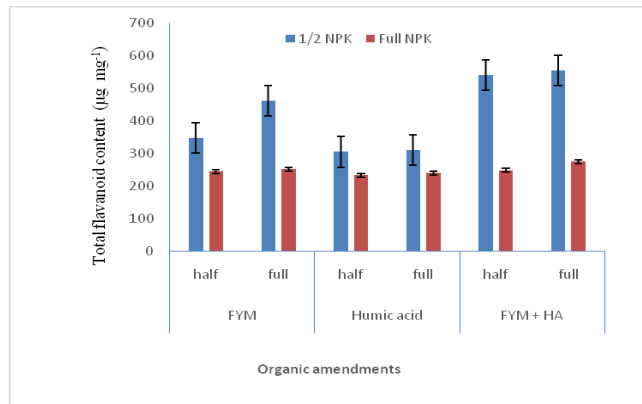


Figure 6: Total flavanoid content (µg mg⁻¹) of chamomile as affected by integration of inorganic fertilizer with farmyard manure and humic acid

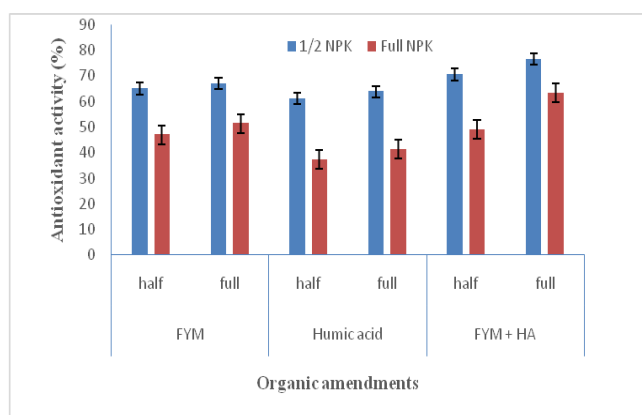


Figure 7: Antioxidant activity (%) of chamomile as affected by integration of inorganic fertilizer with farmyard manure and humic acid

Secondary metabolite production and antioxidant activities

Data regarding secondary metabolite production and antioxidant activities are presented in table 5. Secondary metabolite production and antioxidant activities of chamomile were statistically ($p \leq 0.05$) affected by the integrated nutrient management. The interaction between NPK \times OA (organic amendments) were found significant & shown in figures 5, 6 & 7.

Regarding mean values, higher concentration for total phenolic content (8 mg g⁻¹ DW), total flavonoid content (554.4 µg mg⁻¹ DW) and antioxidant (76.4% DW) were noticed in plants fertilized with half NPK + full FYM + full HA closely pursued by 7.82 mg g⁻¹ DW, 542 µg mg⁻¹ DW and 70.5% DW in treatment with combination of half NPK + half FYM + half HA, respectively.

A decreasing pattern was observed in stated parameters i.e. 5.27 mg g⁻¹ DW, 274.80 µg mg⁻¹ DW and 57.81% in control. However, further decrease was recorded in plants supplied with full NPK i.e. TPC (3 mg g⁻¹ DW), TFC (214.3 µg mg⁻¹ DW) and antioxidant (32.4% DW). It was noticed that with increasing NPK levels the concentration of phytochemicals and biological activities decreased but with increase in organic manure increased the concentration and activities.

Discussion

Growth attributes of chamomile were statistically affected by integrated nutrient management. The findings of many researchers supported the present results. Tallest plants, a maximized number of leaves and flowers per plant, enlarged flower and stem diameter and greater hundred seed weight and root length were documented (Baloch *et al.*, 2010 and Lehri *et al.*, 2011).

The reason might be the participation of NPK in plants. Organic fertilizer slowly liberated nutrients and might not fulfill plant demands, so from inorganic sources of nitrogen is instantly available to plants at primary stage, thus improved vegetative growth (Heeb *et al.*, 2005). Nitrogen is supplementary in high photosynthetic activity, vigorous growth, branching and leaf production

(Inamullah and Khan, 2014). Whereas, phosphorus is recognized as vital component in nucleoprotein, participated in imperative role in cell division (Inamullah and Khan, 2014) and deficiency might subdue growth of crop (Inamullah and Khan, 2014). Likewise, numerous investigators have shown that integration of inorganic and organic manures statistically improved the vegetative attributes of plants (Alamgir *et al.*, 2011). The efficiency of NPK was enhanced by the integration of NPK with humic acids. It was documented that the HA incorporation in soils boosted growth attributes of roots (origination, increase, branching of root hairs) and roots became more efficient in nutrient grasping hence the nutrient levels in plants were increased (Atiyeh *et al.* 2002). Khattak and Dost (2010) also documented that improved growth attributes were observed in plant through integration of HA with synthetic fertilizers which positively contributed in nutrient uptake and growth of crop. It might be linked with the proficiency of HA to make favourable soil biological medium by endorsing water holding capacity, enzymatic and microbial activities that eventually boosted uptake of nutrients and crop growth. Growth characters were further increased by conjunction of NPK and FYM. These results were in harmony with Bandyopadhyay *et al.* (2003) who documented that the better efficacy of soybean

Table 5: Total phenol and total flavonoids of chamomile as affected by integration of inorganic fertilizer with farmyard manure and humic acid

Treatment	Total phenols (mg g ⁻¹ DW)	Total flavonoids (µg mg ⁻¹ DW)	Antioxidant Activity (%)
Control	5.27 f	274.80 f	57.81 f
NPK	3.0 l	214.3 m	32.4 m
FYM	4.5 h	264.6 h	52.3 h
HA	4.9 g	268.1 gh	55.8 g
1/2 NPK+1/2 FYM	6.4 d	347.3 d	65.0 cd
1/2 NPK + 1/2 HA	5.7 e	304.8 e	61.2 ef
1/2 NPK + 1/2 FYM+1/2 HA	7.82 b	542 b	70.5 b
Full NPK + 1/2 FYM	4.0 ij	244.9 jk	47.0 j
Full NPK + 1/2 HA	3.4 k	233.1 l	37.2 l
Full NPK + 1/2 FYM + 1/2 HA	4.2 i	248.5 ij	49.1 ij
1/2 NPK + full FYM	6.9 c	462.4 c	66.9 c
1/2 NPK + full HA	6.0 e	310.2 e	63.8 d
1/2 NPK + full FYM+Full HA	8.0 a	554.4 a	76.4 a
full NPK + full FYM	4.3 hi	251.9 i	51.4 hi
Full NPK + Full HA	3.8 j	240.3 k	41.4 k
Full NPK + full FYM + full HA	5.8 e	274.3 fg	63.2 de
LSD at α 0.05	0.30	6.51	2.50
Interaction			
NPK × OA	*	*	*
T × Y	NS	NS	NS

Considerably dissimilar means were symbolized by different alphabets. 5% significance level is shown by * whereas NS means nonsignificant. NPK × OA (organic amendments) interaction between NPK levels and organic amendments whereas T×Y is interaction between treatment and years.



under NPK+FYM was attributed to boosted growth of root and proficient consumption of rain. Alone application of inorganic or organic fertilizers could not give sustainability of organic matter in soil and productivity (Prasad, 1996).

As regards vegetative growth characters, year was found a source of variation during 2016-2018. The increment in growth attributes in 2017-2018 as linked to 2016-2017 might be credited to optimal rainfall and suitable weather conditions that positively improved growth of crop. Sufficient water availability might have stretched the roots in all directions by increasing carbohydrates transfer to roots and absorption of more nutrients ultimately resulted in enhanced crop growth (Kramer, 1975). Optimal water was needed by crops to dissolve and absorb the nutrient and was also imperative in metabolic pathways which were linked to growth and development of crop (Hartmann *et al.*, 1981).

Essential oils are highly volatile formed in glandular trichomes and characterized by concentrated and intense aroma (Francoise, 2003). Current results were in similarity with many other researchers who depicted that cattle manure improved quality of medicinal plants by boosting the contents and concentration of essential oil such as sage (Kocabas *et al.*, 2010), basil (Biasi *et al.*, 2009) and onion (Yassen & Khalid, 2009). Similarly, Rahman *et al.* (2015) reported that integrated nutrient management significantly increased herb yield essential oil content in Kosi "variety of Peppermint (*Mentha piperita* L.).

Nutrient availability enhanced with the use of FYM which enhanced vegetative growth and flower development of plants (Hercencia *et al.*, 2011) ultimately resulted in highest yield. Chamomile flower production was significantly increased through integrated nutrient management as associated to unfertilized plots. Furthermore, growth characters and production in strawberry were statistically enhanced through optimum and appropriate incorporation of fertilizer (Ogendo *et al.*, 2008).

Favourable soil and environmental conditions (increased organic matter, optimal temperature and higher rain) recorded in 2017-2018 might be the reason for greater yield. The higher moisture of soil because of higher rain boosted the captivation of nutrients ultimately augmented production. During 2016-2017 incorporation of organic manure resulted in a higher concentration of organic matter in the soil that might be the reason which had higher residue effect and amplified production of the subsequent crop (Makinde and Ayoola, 2008).

Secondary metabolite production and antioxidant activities

Increasing and decreasing trends were observed during the phytochemicals determination. It was observed that plants in control treatment possessed higher concentration of TPC and TFC than at the optimum dose of NPK. Minimum concentration of TPC and TFC was found in plants receiving NPK at 100:50:50 kg ha⁻¹. Mineral nutrition significantly affected primary and secondary metabolic rate in higher plants (Caretto *et al.*, 2015). Investigated results were supported by (Glynn *et al.*, 2008). It was illustrated that nutrient (phosphorous and potassium) deficient medium have been documented to up-regulate the quantities of polyphenolic components either as extant pool or through prompting their *de novo* synthesis.

It might be due to nutritional stress which acts as stimulator to increase synthesis of phenolic compounds (Caldwell *et al.* 2003) to defend themselves in stressed conditions. It might be due to CNB (carbon nutrient balance) assumption, which illustrated that nutrient stress caused the build-up of excessive carbon which boosted synthesis of CBSM (carbon-based phytochemicals) and their predecessors (Yongke *et al.* 2005). Progressive increase in carbon containing structure for ammonium absorption might have activated shikimic acid pathways which in response boosted the synthesis of secondary metabolites (Fan *et al.* 1998).

Decrease in sink size resulted in reduction of carbohydrate translocation to other parts of plant (Reddy *et al.* 1996) and additional carbohydrates might be directed to secondary metabolites production (Tognetti and Johnson, 1999).

DPPH is a rapid and sensitive method to evaluate the antioxidant capacity of plant extracts. It is based on the decolourization of DPPH in the presence of antioxidants in the tested sample. The colour change from purple to yellow shows the strong antioxidant capacity of the sample. Oxidative stress is caused by free radicals which are reactive oxygen species (ROS) formed as a by-product of cellular aerobic respiration (Yanai *et al.*, 2008). The antioxidant ability of phenolic components occurs mainly through a redox mechanism and allows the components to act as singlet oxygen quenchers, metal chelators, hydrogen donors and reducing agents (Ahmad, 2017).

The antioxidant activity was found to be highest under organic fertilization than inorganic fertilization. These results were supported by the findings of (Ibrahim *et al.*, 2011) and documented strong positive relationship



between total phenolics, flavonoid compounds and antioxidant activity.

Conclusions and recommendations

It was observed that a maximum survival percentage in plants was found in all plots irrespective of treatments. Growth and qualitative traits were statistically affected by various treatments. During the growth period of chamomile maximum production (growth, essential oil and yield) were observed in plants fertilized with NPK at 100:50:50 + FYM at 20 t ha⁻¹ + HA at 5 kg ha⁻¹. Minimum growth attributes and flower production were recorded in plants grown in unfertilized plots (control). Regarding secondary metabolites production and anti-oxidant activities maximum concentrations were found in plants supplied with NPK at 50:25:25 + FYM at 20 t ha⁻¹ + HA at 5 kg ha⁻¹ closely followed by concentrations in plants treated with NPK at 50:25:25 + FYM at 10 t ha⁻¹ + HA at 2.5 kg ha⁻¹. It is recommended that chamomile should be cultivated in Azad Jammu and Kashmir because it is well adapted to that area and plants should be treated with NPK at 100:50:50 + FYM at 20 t ha⁻¹ + HA at 5 kg ha⁻¹ for maximum growth and production. Plant should be grown in NPK at 50:25:25 + FYM at 10 t ha⁻¹ + HA at 2.5 kg ha⁻¹ for higher secondary metabolite production and antioxidant activities.

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