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INTERACTIVE EFFECT OF HUMIC ACID AND FARMYARD MANURE ON SOIL HEALTH AND MICROBIAL ACTIVITY IN CALCAREOUS SOIL

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Abstract

Humic acid (HA) improves soil solids surface chemistry and thereby optimizes biological activity and fertility of soil. However, its interactive effect with farmyard manure (FYM) under calcareous soil is not fully explored. Therefore, a laboratory incubation experiment (1^{st}) was conducted to investigate effect of various levels of lignitic coal derived humic acid (0, 100 and 200 mg kg^{-1} soil) applied alone or in conjunction with 10 g FYM kg^{-1} on soil microbial activity and nutrients availability for 79 days. In the 2^{nd} experiment, the same soil was remoistened and treated with lower levels of HA (0, 2, 4, 6, 8 and 10 mg kg^{-1}) for 14 days to further verify its effect on soil microbial activity. The combine application of HA and FYM significantly increased CO_2 release except at 2^{nd} day, however, application of 100 and 200 mg HA produced non-consistent supremacy over each other at different incubation intervals. Combine use of HA and FYM consistently accelerated the rate of CO_2 production over all incubation intervals as compared to the sole application of HA or control. Cumulative release of CO_2 also showed that application of HA either as 100 or 200 mg produced higher (1014 and 1035 mg CO_2 kg^{-1} respectively) as compared to control (918 mg kg^{-1}) at 79^{th} day. Integrated use of HA+FYM further amplified cumulative CO_2 releases as compared to control or alone HA at both levels. In the second experiment, application of HA increased CO_2 release with increasing doses up to 6 mg HA kg^{-1} only at day 4 of incubation while its effect was non-significant for the rest of incubation interval. The integrated application of HA and FYM significantly improved post incubated soil pH, Organic matter and NPK content compared to their sole application. Therefore, it can be concluded that the application of FYM further amplify the positive effect of humic acid on soil health and shall be adopted.

Key words: Humic acid, Farmyard manure, Cumulative CO_2 releases, Incubated soil, Microbial activity.

Introduction

Calcareous soils contain large amount of $CaCO_3$ and cover more than 30% of the Earth's surface (Bolan *et al*., 2023). High $CaCO_3$ adversely affect the physical and chemical properties of these soils (FAO, 2016). Low water-holding capacity, high pH and infiltration rate, low CEC, low organic matter (OM) and clay contents, poor structure, nutrient loss by leaching or deep percolation, surface crusting and cracking, and N fertilizer loss are only a few of the difficulties associated with cultivating these soils, nutritional imbalances, especially P and micronutrients (El-Hady & Abo-Sedera, 2006). However, organic matter/substances and the availability of N can mitigate the high carbonate content related adversities in calcareous soils.

Organic substances release essential nutrients upon decomposition (Andriamananjara *et al*., 2019). Farmyard manure (FYM) has a beneficial effect on soil biota, microbial activity and nutrient transformation, chemical properties like CEC, pH, nutrient chelation, solubility and physical properties like porosity, bulk density, structure and movement of states and energy (Melero *et al*., 2009). It enhances the bio-availability of nutrients, reduce losses, accelerate microbial activities and release such enzymes and hormones that ultimately improve crop yield and quality (Adnan *et al*., 2018).

Humic acid (HA) has been used as a soil conditioner and fertilizer in agriculture on a small scale. Humic compounds have been shown to have important effects on plant development and soil structure. Their application in right quantities can help plants and roots to develop faster (Minhas *et al*., 2024). Commercial nutrient-containing products known as humic substances (HS) enhance soil fertility, boost nutrient availability, lessen the adverse effects of chemical fertilizers, and eliminate harmful NO_2 and NO_3 ions from the soil, all of which improve plant performance (Osman & Rady, 2012; Burton *et al.*, 2003). According to Sangeetha *et al*., (2006), they make up the majority of the soil's organic matter. By altering the soil's chemical and biological composition, they restore soil fertility. They also enhance the performance and biomass of plants. By improving soil structure and qualities like aggregation, aeration, permeability, water-holding capacity, and the availability and movement of micronutrients, HS can indirectly raise soil fertility (Cimrin & Yilmaz, 2005). They have the direct ability to influence the mechanisms involved in their absorption and movement into plant tissues (Nardi *et al*., 2002). Furthermore, by forming complex forms or chelating agents with metallic cations, HS can alter the solubility of numerous nutrients (Ouni *et al*., 2014). According to Jamal *et al*., (2023), HS can interact with P in the soil to boost plant uptake and decrease fixation. Under conditions of salinity and high carbonate content, the HA can enhance plant performance and the uptake of macro and micronutrients by plants (Katkat *et al*., 2009). By providing nutrients to developing plants, HS can improve the soil's fertility and yield (Osman & Rady, 2012; Brye *et al.*, 2006). Barron and Humic acid increases the activity of microbes, cation exchange capacity and forms chelates with other nutrients, buffers soil pH (Julie & Bugbee, 2006). Sarlak *et al*., (2024) also reported that humic acid serves as catalyst in increasing microorganism activities in soil.

Almost 90% of Pakistan's soils are calcareous in nature, alkaline in reaction, low in organic matter and deficient in several essential nutrients. The main practice to correct the nutrients deficiency in Pakistan is applying the chemical fertilizers. The price of chemical fertilizers is increasing with exponential rate posing the main obstacle in balanced and economical fertilization in Pakistan. Huge amount is spent on the import of chemical fertilizer every year. Along with higher input costs, continuous use of inorganic fertilizers may not be sustainable and can cause soil degradation and hazardous effects of environment (Sarlaki *et al.,* 2024). Hence chemical fertilizer needs to be supplemented with organic manures to produce on sustainable basis for ever increasing population and keep soil and environment from further decay. We hypothesized that, the combine application of humic acid with FYM has preservative effect in promoting the availability of nutrients, production and yield of different crops. However, their performance depend on soil and climatic conditions. Therefore, in the present study, influence of various levels of HA applied alone or in mixture with organic manure on microbial activity and soil fertility was investigated under calcareous conditions to verify their beneficial role in crop production.

Material and Method

Soil sampling: The fresh composite soil sample was collected from top surface (0-15 cm deep) from the new developmental farm the University of Agriculture Peshawar, Khyber Pukhtoonkhwa, Pakistan. The fresh soil sample was shade dried, and sieved (mesh size < 2mm). The moisture content in soil was determined for the purpose of correction of data on dry mass basis. The experimental soil was non saline (0.36 dSm^{-1}) alkaline (7.6 pH), low in organic matter (1.14%), N (55.1 mg kg-1) and P (3.20 mg kg-1) while, was sufficient in K (180.6 mg kg-1). The experimental site is semi-arid (300– 500 mm rainfall $year^{-1}$) in nature where more than 60% rainfall occurs in summer season (Adnan *et al*., 2018).

Experimentation: In 2016, two laboratory incubation experiments were carried out in the Department of Soil and Environmental Sciences' Soil Microbiology laboratory at the University of Agriculture, Peshawar, Pakistan. A basic completely randomized design (CRD) with three replications was used for both trials. In order to confirm their advantageous role in crop production, the purpose of these experiments was to assess the effects of different concentrations of HA applied either alone or in combination with organic manure on microbial activity and soil fertility under calcareous conditions. Therefore, in 1^{st} experiment, a 50 g air dried collected non-saline, calcareous soil (already collected and processed) was taken in a 500 ml conical flask and treated with HA at the rate of 0, 100, 200 mg kg^{-1} with and without FYM (20 t ha^{-1} or 10 g kg^{-1}). Basal dose of N and K was applied to all treatments at the rate of 60 mg N and 30 mg K_2O kg^{-1} as urea and sulfate of potash. Prepared flasks along with three blanks were further processed for CO_2 evaluation according to the procedure of Horwath & Paul (1994) and incubated at 28±2ºC for 79 days. Microbial activity as index for CO_2 release from soil was measured on 2, 5, 11, 21, 36, 52, 79 day of incubation. Similarly, another experiment was conducted with lower levels of humic acid (0, 2, 4, 6, 8 and 10 mg HA kg^{-1}) for a period of 14 days to see effect of lower levels of HA on soil microbial activity at day 4, 8, 11 and 14 of incubation. The distilled water was spared on soil on the day of reading to balance the moisture loss by maintaining the initial weight of flask containing soil.

Laboratory analysis: The conical flask with a capacity of 500 mL were used, to take a 50 g HA and FYM treated soil as per proposed treatment structure. A 5 ml of 0.3 N NaOH were taken in a vial and properly sealed in a conical flask, and incubated at 28ºC for 0, 3, 5, 11, 21, 36, 52, and 79 days for the first experiment and 4, 8, 11 and 14 days for the second experiment. The vial was taken out at each incubation period and titrated against 0.1 N HCl in the presence of 10 ml 1 M BaCl2 solution using phenolphthalein as indicator. The amount of HCl consumed in titration was used to calculate the amount of CO_2 in each flask. The CO_2 produced was calculated by difference between sample and blank (without soil) readings by the following formula (Frioni, 1990):

$$CO_2 \text{ release (mg kg} - 1\ CO_2 - 1 = \frac{(\text{Blank} - \text{Sample}) \times E}{W \times \text{Days}}$$

where blank and samples are is average HCl volume used in the titration of the blanks and samples, respectively, W is weight of dry soil (g), and E is CO2 equivalent.

Changes in soil pH were measured in 1:5 suspensions (McClean, 1996), organic matter by using $K_2Cr_2O_7$ as an oxidizing agent as decried by (Nelson & Sommer, 1996) AB-DTPA extractable P and K by the method (Soltanpour & Schwab, 1977) and mineral N by Kjeldahl distillation method (Mulvaney, 1996). These parameters were determined in the soil at the end of incubation to see the effect of humic acid and farmyard manure.

Statistical analysis

Analysis of variance (ANOVA) was carried out according to complete randomized design using MSTATC package (Russel, 1989). Means were also compared using the least significant difference (LSD) test (Steel & Torrie, 1980).

Results

Rate of CO_2 release (mg kg^{-1}) from incubated soil: The release of CO_2 showed significant ($p<0.05$) increase with application of humic acid and FYM expect on day 2nd of incubation where its effect was non-significant (Table 1). The CO_2 release tended to increase non- significantly with 100 and 200 mg Kg^{-1}. However, the conjunctive use of HA+FYM consistently accelerated the rate of CO_2 production over all incubation intervals as compared to sole supplementation of HA or control but supremacy over sole application of FYM was variable at different incubation intervals. At some incubation intervals sole application of FYM produced higher CO_2 release as compared to HA+FYM that might be associated with higher doses of HA. On 5th day of incubation, the conjunctive use of 100 mg HA+FYM and 200 mg HA+FYM resulted in higher CO_2 releases of 35.82 and 35.62 as compared to 32.82 mg CO_2 kg^{-1} d^{-1} with application of FYM alone. Sole application of HA also produced higher rate of CO_2 as compared to control but this was less than FYM or HA+FYM. On day 11th, HA applied alone or in combination with FYM produced higher CO_2 as compared to control but less than sole FYM. Though conjunctive use of HA+FYM produced less CO_2 as compared to sole FYM but the difference was non-significant. On day 21, similar trends were observed. One day 36th, the conjunctive use of HA+FYM resulted significantly ($p<0.05$) higher releases of CO_2 as compared to control and sole application of HA but non-significant to sole FYM. On day 52 and 79 similar trends were observed but this time HA at 200 mg kg^{-1} + FYM produced lower CO_2 as compared to alone FYM or 100 mg HA+FYM. Comparing changes of CO_2 release over incubation intervals, it was observed that initially the rate of CO_2 releases was increased up to day 21 for control FYM and day 36 for 10 g FYM kg^{-1} soil and then declined with time up to 79th day of incubation.

Cumulative CO_2 release (mg kg^{-1}): The results of cumulative release of CO_2 showed discrete differences among various treatments at the end of 79th day of incubation period. Application of HA either as 100 or 200 mg kg^{-1} soil produced higher CO_2 release of 1014 and 1035 mg kg^{-1}, respectively as compared to 918 mg kg^{-1} in case of no-HA (control) (Fig. 1). Conjunctive use of HA+FYM also showed remarkable increase in CO_2 releases as compared to control (0 HA and 0 FYM) or alone HA at both levels, however, these releases were less as compared to sole application of FYM. The highest cumulative CO_2 release (mg kg^{-1}) was observed in sole FYM treated soil while the lowest was observed in control. Similarly, under the 2nd incubation study the highest cumulative Co2 was recorded under 6 mg HA ha^{-1} while the lowest were recorded under control as shown in Fig. 2.

Release of CO_2 from incubated soil treated with lower doses of HA: In the 2nd incubation study where CO_2 release was evaluated with lower doses as shown in table 2. The results showed that application of HA consistently increased CO_2 release from soil with increasing doses up to 6 mg HA kg^{-1} at day 4th of the incubation while on day 8, 11 and 14 its effect was non-significant. Similarly, the highest cumulative release of CO_2 as 594 mg kg^{-1} was recorded at 6.0 mg HA kg^{-1} as compared to control (502 mg kg^{-1}) at the end of 14 days of incubation. These results confirmed the influential effect of HA on microbial activates and suggested that lower doses of HA upto 6 mg kg^{-1} soil equivalent to 12 kg ha^{-1} was the most appropriate doses of HA for improving the microbial activity in soil.

Changes in soil pH, SOM, mineral N and AB-DTPA extractable P and K: The analysis of variance indicated that integrated application of HA and FYM significantly affected soil pH, organic matter, mineral N and ABDTPA extractable P and K as compared to control (0 HA and 0 FYM) (Table 3). The sole application of HA at the rate of 200 mg kg^{-1} soil significantly lowered soil pH from 7.72 in control to 7.51 while there was no effect of HA on soil pH where FYM was applied at the rate of 10 g kg^{-1} soil. There organic matter content didn’t show significant response to HA application under the same level of organic matter, however under the both control and 10 g FYM kg^{-1} soil organic matter content was increased with increasing level of HA. Application of HA at the rate of 200 mg and 100 mg per kg soil along with + 10g FYM kg^{-1} soil resulted higher soil organic matter of 1.57 and 1.52% respectively as compared to 1.41% in sole FYM. Application of HA at 100 and 200 mg kg^{-1} soil produced OM of 1.15 and 1.14% which is similar to 1.12% of the control. The conjunctive use of HA+FYM produced higher organic matter content than alone application of FYM and HA treatments. The higher mineral N and ABDTPA extractable P and K of 136.2, 12.84 and 194 mg kg^{-1} respectively were observed in treatments receiving 200 mg HA + FYM as compared to 104.7, 8.87 and 183 mg kg^{-1} soil in control respectively. The P concentration showed little changes with HA applied. The highest P as 14.10 mg kg^{-1} was recorded in sole FYM treatment which was statistically at par to 100 and 200 mg HA kg^{-1} + 10 g FYM kg^{-1} soil while significantly higher than 8.87 mg kg^{-1} in control. Combined application of HA+ FYM significantly improved soil mineral N, ABDTPA extractable K and organic matter while didn’t affect ABDTPA compared to sole application of FYM.

Table 1. Effect of HA and FYM on rate of CO_2 at given intervals released from soil incubated for 80 days at 28±2°C.

Treatment (kg^{-1} soil)		Incubation time (days)						
		2	5	11	21	36	52	79
HA (mg)	FYM (g)	CO_2 Release (mg kg^{-1} d^{-1})						
0	0	63.50	20.61 b	16.81 b	9.91 c	6.95 b	12.57 b	8.29 c
100	0	60.37	23.02 b	18.70 b	10.38 c	8.86 b	12.33 b	10.30 bc
200	0	56.93	25.22 b	17.33 b	11.94 c	8.52 b	13.72 b	10.17 bc
0	10	68.19	32.82 a	24.37 a	17.57 a	15.13 a	17.99 a	13.61 a
100	10	66.32	35.82 a	23.42 a	16.29 ab	17.38 a	17.93 a	11.87 ab
200	10	66.00	35.62 a	22.58 a	13.63 bc	17.44 a	15.82 ab	12.27 ab

Means with different letter(s) are significantly different ($p<0.05$). HA, FYM, OM, N, P and K represent humic acid, farmyard manure, organic matter, nitrogen, phosphorus and potassium, respectively

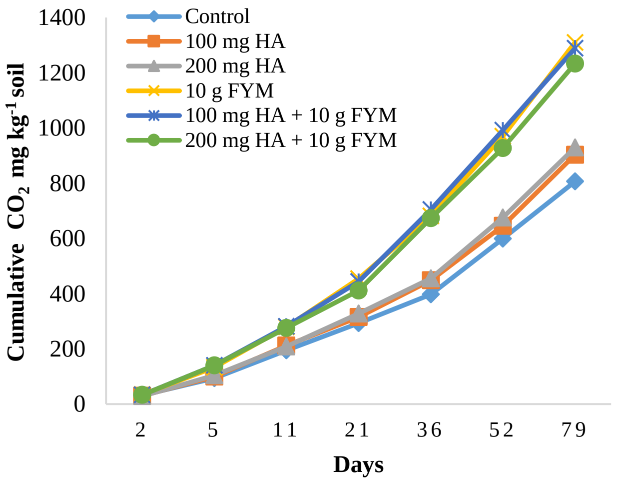

Fig. 1. Cumulative CO_2 released from soil treated with HA and FYM during incubation at room temperature (28±2°C). HA and FYM represents humic acid and farmyard manure, respectively.

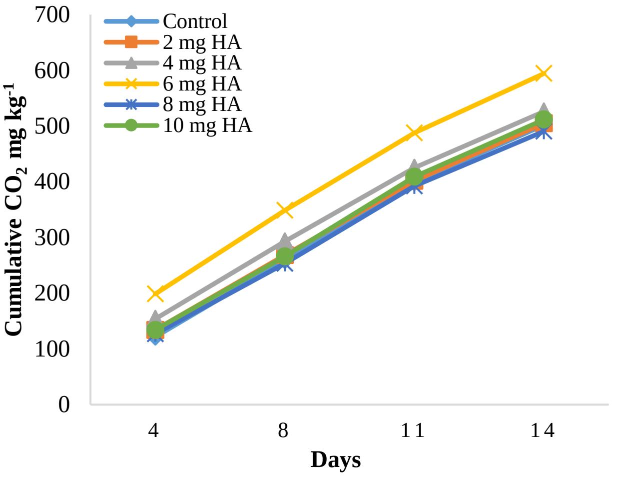

Fig. 2. Cumulative CO_2 (mg kg^{-1}) released from soil treated with lower doses of HA during incubation at room temperature (28±2°C). HA stands for humic acid.

Table 2. Release of CO_2 from soil treated with lower doses of HA.

HA (mg kg^{-1})	Incubation interval (days)			
	4	8	11	14
	CO_2 (mg kg^{-1}d^{-1})			
0	30.39 b	34.38	46.75	34.09 a
2	33.52 b	33.46	44.46	34.55 a
4	38.43 b	34.83	44.00	33.63 a
6	49.63 a	37.58	46.29	35.47a
8	31.73 b	31.63	46.29	32.70 a
10	33.52 b	33.00	47.67	34.09 a

Means with different letter(s) are significantly different ($p<0.05$). HA, FYM, OM, N, P and K represent humic acid, farmyard manure, organic matter, nitrogen, phosphorus and potassium, respectively

Discussion

The soil microbial respiration is the first and the most often used index that reflects the value of emitted CO_2 during the respiratory process of the microorganisms in the soil and shows their number as well as their metabolic activity (Parastesh *et al*., 2019; Smitha *et al*., 2019). Our finding indicated that humic acid improved the biological properties of soil as indicated by the enhanced releases of CO_2 during incubation. The supplementary experiment using lower doses of HA showed that CO_2 release was increased with increasing doses of HA up to 6.0 mg HA ha^{-1} at all incubation intervals. The combine application of HA+FYM produced higher rate and cumulative CO_2 releases as compared to sole application of HA or control (0 HA and 0 FYM) but supremacy over sole FYM was inconsistent at different incubation intervals. These results are in accordance with Parastesh *et al*., (2019) Sharif, (2002), and Dost & Khattak (2008) who reported increases in the activities of microbes with humic acid supplementation. Ampong *et al*., (2022) reported that the biotic properties of soil got improved with humic acid application. Though a very little amount of humic acid (about up to 2-4 kg HA ha^{-1}) is usually recommended as compared to the dose of 100 and 200 mg HA kg^{-1} which are equivalent to 200 and 400 kg HA ha^{-1}, respectively. The decrease in microbial activity with HA+FYM as compared to sole FYM as evident from cumulative release of CO_2 (Figs. 1 & 2) was further investigated taking lower levels of HA as 0, 2, 4, 6, 8 and 10 mg HA kg^{-1}. Adnan *et al*. 2018 conducted an incubated study and reported that emission of CO_2 was more pronounced in soils treated with organic sources (PM and FYM) of phosphorus as compared with mineral sources (SSP and RP) of phosphorus. Yang *et al*. (2021) observed a very high level of CO_2 emissions during week 1 of incubation. Decreased soil respiration over time may be due to a reduction in digestive processes and a lack of easily decomposing nutrients in the soil that support a limited number of soil organisms (Adnan *et al*., 2018; Bhuma *et al*., 2003).

HA application with FYM declined the efficiency of sole FYM which might be associated to higher levels of humic acid as 100 mg HA kg^{-1} equivalent to 200 kg HA ha^{-1} while HA is usually applied to soil in 2 to 4 kg ha^{-1} (Sharif, 2002, Sharif *et al*., 2003, Khattak & Muhammd, 2008; Chatterjee *et al*., 2011). Janssens & Luyssaert (2009) discussed that soil fertility has a profound effect on carbon dioxide (C) in the global ecosystem. Sathiya *et al*. (2003) reported a linear trend in the release of N up to 20 and P and K up to 40 kg HA ha^{-1}. Similarly, Sarir, (1998) and Sharif *et al*., (2002) also advocated for use of lower doses of HA.

Soil pH showed a non-significant declining trend with HA application as compared to control or sole application of FYM. However, with application of FYM the soil pH was significantly reduced regardless of HA application. Likewise, Jamal *et al*., (2024) found that the addition of FYM decreased soil pH in calcareous sandy soil. The soil pH could have been reduced due to the chemical oxidation and microbiological decomposition of FYM in soil, which produced acidic compounds that help reduce soil pH. The production of organic acids (amino acid, glycine, cysteine, and humic acid) during the mineralization (ammonization and ammonification) of organic materials by heterotrophs and nitrification by autotrophs can also cause a decrease in soil pH (Kumar *et al*., 2020).

In our case, the addition of FYM also increased SOM. Other researchers also found that FYM not only reduced the oxidation stability of SOM but also improved the SOM content of the soil up to 1.2–2.9 kg ha^{-1} (Li *et al*., 2017; Ding *et al*., 2020). The increase in SOM with

FYM treatment may be partially due to the input of organic matter found in the FYM (Li *et al*., 2017; Rehim *et al*., 2020), although the increase in SOM. We generally observed a increase in plant available N, P (AB-DTPA) and P with the addition of humic acid and FYM. In general, FYM application has appreciable and dynamic impacts on the chemical fractions of P because P from FYM gradually turns into available forms over time (Ma *et al*., 2020). The increase in available NP and K in our study might be due to the release of significant quantities of CO2 during FYM decomposition (Andriamananjara *et al*., 2019) and the complexing of cations such as Ca+2, thus reducing their fixation in calcareous soils (Fixen & Bruulsema, 2014; Jamal *et al*., 2018; McMullen *et al.*, 2015). Furthermore, the FYM contains organic acids, which are known to increase nutrients solubility (Hopkins, 2015). HA being a poly functional molecule form chelates with nutrients and keep them in solution (Davies *et al*., 2001). HA treated soils produce additional CO_2 that should be linked to improve the dissolution of Ca-apatite by carbonic acid formed due to respiration of microbial activities (Adnan *et al*., 2019).

Table 3. Changes in soil pH, organic matter, mineral N and AB-DTPA extractable P and K as influenced by HA and FYM at day 79 of incubation.

HA (mg kg^{-1})	FYM (g kg^{-1})	pH	OM (%)	N	P	K
				mg kg^{-1}		
0	0	7.72 a	1.12 a	104.70 c	8.87 b	183 b
100	0	7.54 ab	1.15 a	109.15 c	9.17 b	187 b
200	0	7.51 b	1.14 a	119.93 b	8.99 b	181 b
0	10	7.70 ab	1.41 b	128.60 a	14.10 a	187 ab
100	10	7.67 ab	1.52 b	130.33 ab	13.49 a	187 ab
200	10	7.67 ab	1.57 b	136.20 a	12.84 a	194 a

Means with different letter(s) are significantly different ($p<0.05$). HA, FYM, OM, N, P and K represent humic acid, farmyard manure, organic matter, nitrogen, phosphorus and potassium, respectively

Conclusions

The integrated application of Humic acid along with farmyard manure improved the fertility and microbial activity of soil. The supplementary experiment using lower doses of HA didn't show significant effect over CO_2 release except at day 4th of incubation where significantly higher CO_2 was released at 6.0 mg HA ha^{-1}. The conjunctive application of HA+FYM produced higher rate and cumulative CO_2 releases as compared to sole application of HA or control but supremacy over sole FYM was inconsistent at different incubation intervals. Soil pH decreased, SOM along with mineral N, and AB-DTPA extractable P and K increased with HA at the end of 79 d of 1st incubation experiment. Conjunctive use of HA+FYM showed increases in N and K but declining trend in AB-DTPA extractable P and K was shown. Further experiments on conjunctive use of lower levels of HA with FYM are suggested for various soils to confirm the results.

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