The Pharma Innovation Journal 2023; 12(9): 847-551

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(9): 847-851

www.thepharmajournal.com
Received: 16-05-2023
Accepted: 20-06-2023

Effect of biochar, humic acid, microbial consortia on the exchangeable Ca and Mg contents of soil cultivated with marigold

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Abstract
The present investigation was carried out at the Horticultural Research Station, Mahanandi, Dr. Y.S.R. Horticultural University during the summer season of 2021 and 2022. The experiment was carried out with three factors and two replications in a randomized block design with factorial concept to study their individual and combined influences on the exchangeable Ca and Mg contents of soil after harvest of flowers in marigold. Among the individual treatments, soil application of biochar @ 10 t ha^{-1}, humic acid @ 4 kg ha^{-1} and *Azospirillum* @ 5 kg ha^{-1} in combination with PSB @ 5 kg ha^{-1} and *Bacillus spp.* @ 5 kg ha^{-1} recorded significantly highest exchangeable Ca and Mg contents of soil after harvest of flowers during 2021, 2022 and the pooled data mean. Among the two combination treatments, soil application of graded levels of biochar and humic acid, biochar and different microbial consortia, humic acid levels and different microbial consortia recorded significantly highest exchangeable Ca content of soil after harvest of flowers during 2021, 2022 and the pooled data mean, whereas the data were found non-significant with regard to exchangeable Mg content of soil during both the years of study as well as in the pooled data mean. Non-significant differences were noticed among the interaction effects of soil application of graded levels of biochar, humic acid and different microbial consortia with regard to exchangeable Ca and Mg contents of soil during both the years of study as well as in the pooled data mean.

Keywords: Biochar, humic acid, exchangeable, calcium, magnesium, marigold

Introduction

African Marigold (*Tagetes erecta* L.), a proud member of the Asteraceae family, has emerged as a lucrative commercial flower crop in Andhra Pradesh, captivating attention for its diverse applications. Day to day the crop is gaining popularity on account of its flowers used in various ways specially for garland making, veni making and for the extraction of pigments called carotenoids and xanthophylls, secondary metabolites from the petals of flower. To increase the productivity and quality of marigold, there is every need to increase the availability of nutrients in soil to put-up proper growth and development of plant. 'Green Revolution' led to increased use of chemical fertilizers, to obtain higher yields in crops, but gradually deteriorated the soil health specially the physical and chemical properties. Many studies have been conducted so far, on many agricultural and horticultural crops to ameliorate the soil health deteriorated due to indiscriminate usage of chemical fertilizers by using bio-fertilizers thus to cutdown the use and heavy expenditure on chemical fertilizers. The present study embarks on a journey that intertwines the heritage and novelty of using different organic materials in soil. Marigold cultivar 'Bidhan-2' celebrated its tenacity amidst summer's harsh embrace, thus takes center stage. Further, to enrich its flower yield potential, we turned to infuse the organic elements like biochar, humic acid and microbial consortia containing different microorganisms into the soil. This union aimed not only to enhance the marigold flower yield and quality, but also to safeguard the fragile equilibrium of the soil.The present investigation primarily aimed to increase the flower yield and quality in marigold cultivated during summer season under the scarce rainfall zone of Rayalaseema region, Andhra Pradesh *i.e.*, at Nandyal by using a summer tolerant marigold cultivar 'Bidhan-2' in combination with incorporation of organic inputs like biochar, humic acid and microbial consortia in soil. The main aim of the study was to determine the available contents of exchangeable Ca and Mg in soil, as these two secondary elements play a major and critical role in the plant growth and development of marigold.

Materials and Methods

The present investigation was carried out at the Horticultural Research Station, Mahanandi, a constituent research institute of Dr. Y.S.R. Horticultural University near Nandyal, the scarce rainfall zone of Rayalaseema region during the summer season of 2021 and 2022. The marigold variety selected was orange coloured 'Bidhan-2' and the experiment was laid out in a Randomized Block Design with factorial concept and replicated twice. The data were subjected to statistical analysis of variance for randomized block design with factorial concept as suggested by Panse and Sukhatme (1967) [1]. The experimental soil was silty loam in texture, containing Ca (12.00 meq 100^{-1} g soil) and Mg (2.50 meq100^{-1} g soil).Just before transplanting of marigold seedlings, applied the uncharred (rice-straw shreds) material @ 5 tonnes per hectare to all the treatments uniformly and the recommended dose of fertilizers @ 80% in the form of calcium ammonium nitrate, single super phosphate and muriate of potash to all the treatments. The entire dose of phosphorus was applied as a basal dose just before planting, whereas nitrogen and potassium were applied in five splits at an interval of 15 days from the day of transplanting. Graded levels of biochar, humic acid along with microbial consortia in the forms of Arka Microbial Consortium - a product of ICAR-Indian Institute of Horticultural Research, Hesseraghatta and a consortium of different individual microbial cultures *viz.*, *Azospirillum*, Phosphorous Solubilising Bacteria and *Bacillus spp.* (K solubilizing bacteria) were applied just before transplanting of seedlings according to the treatment combinations fixed. The experiment consisted of 18 treatment combinations comprising of rice-straw biochar (BC_0: No biochar, BC_1:5 t ha^{-1} and BC_2: 10 t ha^{-1}), humic acid (HA_1: 2 kg ha^{-1} and HA_2: 4 kg ha^{-1}) and different microbial consortia (MC_0: No microbial consortium, MC_1: Arka Microbial Consortium @ 15 kg $ha^{-1}$and MC_2: *Azospirillum* @ 5 kgha^{-1}+ PSB (*Phosphorus Solubilizing Bacteria*) @ 5 kg ha^{-1}+ *Bacillus spp.*@ 5 kg ha^{-1}). Soil samples were collected in each treatment after harvest of flowers and analysed for exchangeable Ca and Mg contents in soil.The exchangeable calcium and magnesium contents were determined as per the procedure outlined by Hesse (1971) [2].

Results and Discussion

Data pertaining to the influence of graded levels of biochar, humic acid, microbial consortia and their interaction effects on the exchangeable Ca and Mg contents of soil after harvest of flowers in marigold were presented in Table 1 and 2. Significant variation was observed in the data with regard to available Ca and Mg contents of soil in the individual factors during both the years of study as well as in the pooled data mean after harvest of flowers in marigold, whereas the data with respect to combination treatments was found significant during 2nd year and the pooled data mean with respect to Ca content, whereas the data were found non-significant during both the years of study as well as in the pooled data mean of Mg content. Among the graded levels, soil application of biochar @ 10 t ha^{-1} recorded significantly highest exchangeable calcium (20.11, 22.05 and 21.08 meq 100^{-1} g soil respectively during 2021, 2022 and the pooled data mean) and magnesium contents in soil after harvest of flowers (5.99, 6.93 and 6.46 meq 100^{-1} g soil respectively during 2021, 2022 and the pooled data mean) in marigold, whereas no biochar applied in soil recorded significantly lowest exchangeable calcium (14.23, 15.07 and 14.65 meq 100^{-1} g soil respectively during 2021, 2022 and the pooled data mean) and magnesium contents (3.50, 4.33 and 3.92 meq 100^{-1} g soil respectively during 2021, 2022 and the pooled data mean). Soil application of biochar @ 5 t ha^{-1} recorded moderate increase in the exchangeable Ca and Mg contents of soil during both the years of study as well as in the pooled data mean. Based on the critical analysis of these results, it may be concluded that soil application of higher quantities of biochar recorded significant increase in the total exchangeable Ca and Mg contents of soil which might be due to the presence of higher amounts of basic cations present in biochar. Higher cation exchange capacity of biochar helped to hold the positively charged ions and reduced the leaching losses of these nutrients. Lehmann *et al.* (2003) [3] and Chan *et al.* (2008) [4] reported that presence of ash in biochar rapidly released the free bases such as Ca, Mg and K into the soil solution thereby increased the exchangeable bases. Abewa *et al.* (2014) [5] noticed significant increase in the available Ca and Mg contents of soil by application of biochar and reported that it might be due to the dissolution of basic cations in biochar and high surface positive charge which might have decreased the leaching losses of positively charged ions (Sukartono *et al.*, 2011) [6]. Niranjan (2018) [7] also reported significant increase in the exchangeable Ca content of soil by increased level of biochar applied. Jyothishree (2020) [8] observed an increase in the exchangeable Ca content of soil by increased level of cob-rind biochar applied.

In between the two levels, soil application of humic acid @ 4 kg ha^{-1} recorded significantly highest exchangeable Ca (17.77, 19.00 and 18.39 meq 100^{-1} g soil respectively during 2021, 2022 and the pooled data mean) and Mg contents (5.02, 5.88 and 5.45 meq 100^{-1} g soil respectively during 2021, 2022 and the pooled data mean) of soil after harvest of flowers in marigold, whereas soil application of humic acid @ 2 kg ha^{-1} recorded significantly lowest exchangeable Ca (16.44, 17.79 and 17.11 meq 100^{-1} g soil respectively during 2021, 2022 and the pooled data mean) and Mg contents of soil (4.40, 5.28 and 4.84 meq 100^{-1} g soil respectively during 2021, 2022 and the pooled data mean). Based on the critical analysis of these results, it may be concluded that soil application of higher concentration of humic acid exerted higher cation exchange capacity of soil which means it could have bound and retained the positively charged ions like Ca and Mgin soil. Application of humic acid in soil increased the availability of Ca and Mgions by releasing them from the mineral particles and other soil components. Ren *et al.* (2022) [9] noticed an increase in the exchangeable calcium content of soil by application of humic acid.

Among the different microbial consortia applied in soil, *Azospirillum* @ 5 kg ha^{-1} in combination with PSB @ 5 kg $ha^{-1}$and *Bacillus spp.* @ 5 kg ha^{-1} recorded significantly highest exchangeable Ca (17.51, 18.82 and 18.17 meq 100^{-1} g soil and respectively during 2021, 2022 and the pooled data mean) and Mg contents (5.39 meq 100^{-1} g soil in the pooled data mean)of soil after harvest of flowers in marigold, whereas no microbial consortia applied in soil recorded significantly lowest exchangeable Ca (16.61, 17.87 and 17.24 meq 100^{-1} g soil respectively during 2021, 2022 and pooled data mean) and Mg (5.02 meq 100^{-1} g soil in the pooled data mean)contents of soil. Soil application of Arka Microbial Consortium @ 15 kg ha^{-1} recorded moderate increase in the exchangeable Ca and Mg contents of soil during both the years of study as well as in the pooled data mean. Based on the critical analysis of these results, it may be concluded that soil application of different microbial cultures consortium accelerated mineralization of organic residues thus improved the availability of secondary nutrients (Demir, 2020) [10]. Almansour (2018) [11] reported significant increase in the exchangeable Ca and Mg contents of soil by application of organic manures in combination with biofertilizers.

Significant differences were noticed in the interaction effects between graded levels of biochar and humic acid with regard to exchangeable Ca content of soil during 2nd year of study *i.e.,* 2022 as well as in the pooled data mean, but the data were found non-significant during 1st year of study *i.e.,* 2021. The data with regard to Mg content of soil was found non-significant during both the years of study as well as in the pooled data mean. Among the treatments, soil application of biochar @ 10 t ha^{-1} in combination with humic acid @ 4 kg ha^{-1} recorded significantly highest exchangeable Ca content (22.85 and 21.93 meq 100^{-1} g soil during 2022 and the pooled data mean) of soil after harvest of flowers in marigold, whereas soil application of humic acid @ 2 kg ha^{-1} alone recorded significantly lowest exchangeable Ca content of soil (14.69 and 14.26 meq 100^{-1} g soil respectively during 2022 and the pooled data mean). Based on the critical analysis of these results, it may be concluded that soil application of higher quantities of biochar in combination with humic acid exerted significant influence on the exchangeable bases of Ca, which might be due to the high cation exchange capacity of biochar. Chan *et al.* (2007) [12] and Yamato *et al*. (2006) [13] reported high porosity and surface to volume ratio of biochar, which in turn increased the exchangeable Ca content of soil. Abujabhah *et al.* (2016) [14] noticed significant impact of woody biochar on the exchangeable Ca and Mg contents in soil, thus boosted the crop yields (Hussain *et al.* 2017) [15]. Application of humic acid in soil formed the complexes with calcium ions, a process known as chelation thereby these chelates helped to protect calcium from being tied up in insoluble forms, thus made it available to growing plants.

Significant differences were noticed in the interaction effects between graded levels of biochar and different microbial consortia with regard to exchangeable Ca content of soil during 2nd year of study *i.e.,* 2022 as well as in the pooled data mean, but the data were found non-significant during 1st year of study *i.e.,* 2021. The data with regard to Mg content of soil was found non-significant during both the years of study as well as in the pooled data mean. Among the treatments, soil application of biochar @ 10 t ha^{-1} in combination with either of the microbial consortia recorded higher exchangeable Ca content of soil after harvest of flowers without any significant differences between the treatments, whereas no biochar and microbial consortia applied in soil recorded significantly lowest exchangeable Ca content of soil (14.23 and 13.85 meq 100^{-1} g soil during 2022 and the pooled data mean). Based on the critical analysis of these results, it may be concluded that addition of biochar in soil increased the negatively charged surface of soil particles thus attracted the positively charged ions such as Ca and made them available to growing plants. Biochar application might have increased the surface chemistry of soil thus it might have resulted in nutrient retention through cation exchange associated with acidic functional groups formed on biochar surface during the process of oxidation, thus application of biochar retained most of the cations *viz.,* Ca, Mg, K and Na (Clough *et al.*, 2013) [16]. Application of microbial cultures consortium in soil helped in the breakdown of organic matter and mineralized the Ca and Mg ions from the calcium and magnesium compounds in soil. Due to the increased microbial activity and mineralization of calcium compounds in soil, increased the exchangeable Ca content of soil. Verma *et al.* (2017) [17] reported that application of biofertilizers in soil enhanced the conversion of unavailable forms of nutrients present to available forms of nutrients to increase the absorption.

Significant differences were noticed in the interaction effects between humic acid levels and different microbial consortia with regard to exchangeable Ca content of soil during 2nd year of study *i.e.*, 2022 as well as in the pooled data mean, but the data were found non-significant during 1st year of study *i.e.*, 2021. The data with regard to Mg content of soil was found non-significant during both the years of study as well as in the pooled data mean. Among the treatments, soil application of humic acid @ 4 kg ha^{-1} in combination with either of the microbial consortia recorded higher exchangeable Ca content of soil after harvest of flowers without any significant differences between the treatments, whereas soil application of humic acid @ 2 kg ha^{-1} alone recorded significantly lowest exchangeable calcium content of soil (17.44 and 16.79 meq 100^{-1} g soil during 2022 and the pooled data mean). Based on the critical analysis of these results, it may be concluded that soil application of higher concentration of humic acid in combination with either of the microbial cultures consortia recorded significant increase in the absorption, translocation and assimilation of macro, secondary and micro nutrients. Microbial cultures consortium is an organic material that contains living microorganisms which helped in enhancing the nutrient availability. Beaulah *et al*. (2004) [18] reported that application of biofertilizers and bio-stimulants in soil enhanced the availability of nutrients by enriching the humus content of soil.

Non-significant differences were noticed among the interaction effects of soil application of graded levels of biochar, humic acid and different microbial consortia with regard to exchangeable Ca and Mg contents of soil during both the years of study as well as in the pooled data mean.

Based on the critical analysis of these results, it may be concluded that soil application of higher amounts of biochar, humic acid and *Azospirillum* @ 5 kg ha^{-1} in combination with PSB @ 5 kg ha^{-1} and *Bacillus spp.* @ 5 kg ha^{-1} increased the exchangeable Ca and Mg contents of soil after harvest of flowers. Among the two combination treatments, soil application of higher amounts biochar and humic acid, biochar and different microbial consortia, humic acid levels and different microbial consortia noticed the significant impact on the exchangeable Ca and Mg contents of soil, thus boosted the flower yield in marigold. High porosity and increased surface to volume ratio of biocharas well as application of humic acid in soil formed certain complexes with Ca and Mg ions, a process known as chelation thereby these chelates helped to protect the Ca and Mg ions from being tied up in insoluble forms, thus made them available to growing plants thereby increased the flower yield in marigold.

Table 1: Influence of biochar, humic acid, microbial consortia and their interaction effects on exchangeable calcium content of soil after harvesting of flowers in marigold (*Tagetes erecta* L.) cv. 'Bidhan-2'

Exchangeable calcium content of soil (meq100^{-1} g of soil) recorded during experimentation (2020-22)

Treatments	First year (2021)			Second year (2022)			Pooled data mean		
Treatments	HA_1	HA_2	Mean	HA_1	HA_2	Mean	HA_1	HA_2	Mean
BC_0	13.84	14.62	14.23	14.69	15.45	15.07	14.26	15.04	14.65
BC_1	16.26	17.70	16.98	17.42	18.72	18.07	16.84	18.21	17.52
BC_2	19.22	21.00	20.11	21.26	22.85	22.05	20.24	21.93	21.08
Mean	16.44	17.77		17.79	19.00		17.11	18.39	

Treatments	MC_0	MC_1	MC_2	Mean	MC_0	MC_1	MC_2	Mean	MC_0	MC_1	MC_2	Mean
BC_0	13.47	14.38	14.83	14.23	14.23	15.23	15.74	15.07	13.85	14.81	15.29	14.65
BC_1	16.79	16.67	17.48	16.98	17.90	17.81	18.49	18.07	17.34	17.24	17.98	17.52
BC_2	19.57	20.52	20.24	20.11	21.48	22.44	22.24	22.05	20.53	21.48	21.24	21.08
Mean	16.61	17.19	17.51		17.87	18.49	18.82		17.24	17.84	18.17	
Treatments	**MC_0**	**MC_1**	**MC_2**	**Mean**	**MC_0**	**MC_1**	**MC_2**	**Mean**	**MC_0**	**MC_1**	**MC_2**	**Mean**
HA_1	16.14	16.27	16.90	16.44	17.44	17.67	18.25	17.79	16.79	16.97	17.57	17.11
HA_2	17.08	18.11	18.13	17.77	18.30	19.32	19.40	19.00	17.69	18.72	18.77	18.39
Mean	16.61	17.19	17.51		17.87	18.49	18.82		17.24	17.84	18.17	

Treatments	BC_0		BC_1		BC_2		BC_0		BC_1		BC_2		BC_0		BC_1		BC_2	
	HA_1	HA_2	HA_1	HA_2	HA_1	HA_2	HA_1	HA_2	HA_1	HA_2	HA_1	HA_2	HA_1	HA_2	HA_1	HA_2	HA_1	HA_2
MC_0	13.30	13.65	16.29	17.30	18.84	20.30	14.05	14.42	17.45	18.35	20.84	22.13	13.68	14.03	16.87	17.82	19.84	21.21
MC_1	13.97	14.80	15.67	17.68	19.18	21.86	14.83	15.64	16.95	18.68	21.24	23.65	14.40	15.22	16.31	18.18	20.21	22.76
MC_2	14.25	15.42	16.82	18.14	19.64	20.84	15.19	16.30	17.86	19.13	21.70	22.78	14.72	15.86	17.34	18.63	20.67	21.81

Source	S.Em±	CD @ 5%	S.Em±	CD @ 5%	S.Em±	CD @ 5%
BC	0.13	0.40	0.10	0.30	0.09	0.27
HA	0.11	0.32	0.08	0.25	0.07	0.22
MC	0.13	0.40	0.10	0.30	0.09	0.27
BC x HA	0.19	NS	0.14	0.43	0.13	0.39
BC x MC	0.23	NS	0.17	0.53	0.16	0.48
HA x MC	0.19	NS	0.14	0.43	0.13	0.39
BC x HA x MC	0.32	NS	0.25	NS	0.22	NS

Table 2: Influence of biochar, humic acid, microbial consortia and their interaction effects on exchangeable magnesium content of soil after harvesting of flowers in marigold (*Tagetes erecta* L.) cv. 'Bidhan-2'

Exchangeable magnesium content of soil (meq100^{-1}g of soil) recorded during experimentation (2020-22)

Treatments	First year (2021)			Second year (2022)			Pooled data mean		
Treatments	HA_1	HA_2	Mean	HA_1	HA_2	Mean	HA_1	HA_2	Mean
BC_0	3.30	3.71	3.50	4.09	4.58	4.33	3.69	4.15	3.92
BC_1	4.27	5.01	4.64	5.16	5.78	5.47	4.72	5.40	5.06
BC_2	5.65	6.33	5.99	6.59	7.28	6.93	6.12	6.81	6.46
Mean	4.40	5.02		5.28	5.88		4.84	5.45	

Treatments	MC_0	MC_1	MC_2	Mean	MC_0	MC_1	MC_2	Mean	MC_0	MC_1	MC_2	Mean
BC_0	3.23	3.53	3.75	3.50	4.07	4.35	4.58	4.33	3.65	3.94	4.17	3.92
BC_1	4.63	4.39	4.90	4.64	5.47	5.24	5.69	5.47	5.05	4.82	5.30	5.06
BC_2	5.84	5.90	6.24	5.99	6.86	6.80	7.15	6.93	6.35	6.35	6.69	6.46
Mean	4.57	4.60	4.96		5.47	5.46	5.80		5.02	5.04	5.39	
Treatments	**MC_0**	**MC_1**	**MC_2**	**Mean**	**MC_0**	**MC_1**	**MC_2**	**Mean**	**MC_0**	**MC_1**	**MC_2**	**Mean**
HA_1	4.31	4.26	4.64	4.40	5.23	5.10	5.51	5.28	4.77	4.68	5.07	4.84
HA_2	4.83	4.94	5.29	5.02	5.71	5.82	6.10	5.88	5.27	5.38	5.70	5.45
Mean	4.57	4.60	4.96		5.47	5.46	5.80		5.02	5.04	5.39	

Treatments	BC_0		BC_1		BC_2		BC_0		BC_1		BC_2		BC_0		BC_1		BC_2	
	HA_1	HA_2	HA_1	HA_2	HA_1	HA_2	HA_1	HA_2	HA_1	HA_2	HA_1	HA_2	HA_1	HA_2	HA_1	HA_2	HA_1	HA_2
MC_0	3.05	3.42	4.23	5.04	5.65	6.04	3.86	4.29	5.18	5.77	6.65	7.07	3.45	3.85	4.71	5.40	6.15	6.56
MC_1	3.24	3.82	4.08	4.70	5.48	6.32	4.05	4.65	4.96	5.52	6.30	7.31	3.65	4.23	4.52	5.11	5.89	6.81
MC_2	3.60	3.91	4.50	5.31	5.83	6.65	4.36	4.80	5.34	6.05	6.83	7.47	3.98	4.36	4.92	5.68	6.33	7.06

Source	S.Em±	CD @ 5%	S.Em±	CD @ 5%	S.Em±	CD @ 5%
BC	0.13	0.40	0.13	0.39	0.06	0.20
HA	0.11	0.33	0.10	0.31	0.05	0.16
MC	0.13	NS	0.13	NS	0.06	0.20
BC x HA	0.19	NS	0.18	NS	0.09	NS
BC x MC	0.23	NS	0.22	NS	0.11	NS
HA x MC	0.19	NS	0.18	NS	0.09	NS
BC x HA x MC	0.33	NS	0.32	NS	0.16	NS

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