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Plant Growth Promoting Rhizobacteria and Humic Acid Improve Growth and Yield of Organically Grown Canola

Shakeel Ahmad[1], Ihsanullah Daur[1],
Samir Gamil Al - Solaimani[1], Sajid Mahmood[1]
[1]Department of Arid Land Agriculture,
Faculty of Meteorology,
Environment and Arid Land Agriculture,
King Abdulaziz University,
Jeddah, 80208, Kingdom of Saudi Arabia

Mohamed Hussein Madkour[2]
[2]Department of Environmental Sciences,
Faculty of Meteorology,
Environment and Arid Land Agriculture,
King Abdulaziz University, Jeddah 80208,
Kingdom of Saudi Arabia.

***Abstract*— Plant bio-stimulant like plant growth promoting rhizobacteria (PGPR) and humic acid (HA) can boost crop production in organic farming due to their various features. This study was conducted to evaluate the potential of PGPR and HA for improving the effectiveness of cow and poultry manure which are very important nutrient sources. A field trial using canola as test crop was conducted according to split plot design in RCBD arrangement with four replications. Three levels of HA (HA_0= No HA, HA_{10}= 10 kg HA ha^{-1} and HA_{20}= 20 kg HA ha^{-1}) as main plot factor and two levels of PGPR (P_0= without PGPR and P_1= with PGPR) as sub-plot factor were used. At the time of harvesting, data regarding growth and yield parameters such as plant height (cm), number of branches $plant^{-1}$, number of pods $plant^{-1}$, number of seeds pod^{-1}, 1000 seed weight (g) and seed yield (t ha^{-1}) was recorded and statistically analyzed using Statistix 8.1 computer software. It was observed that PGPR and HA improved all the growth and yield parameters. Significant interaction between PGPR and HA was also observed in their effect on 1000 seed weight and seed yield. It is concluded from the results that combined application of both PGPR and HA can improve growth and yield of organically grown canola more than sole application of each partner.**

Keywords—PGPR; humic acid; cow manure; poultry manure; canola

I. INTRODUCTION

Excessive and irrational use of chemical fertilizers and pesticides is putting the agricultural sustainability under great risk by deteriorating soil, water and environmental quality and health [1]. The ill effects of excessive use of fertilizers and pesticide include; pollution of ground water, negative impact on biodiversity, enhanced resistance of pests to chemicals, nutrient imbalance, soil salinization, impaired soil physical health resulting in erosion and soil degradation, reduced food quality due to entry of chemical residues, enhanced greenhouse gas emission and loss of natural habitat [2, 3]. The awareness about the negative impacts and issues generated by modern day agriculture gave birth to a sustainable farming approach called concept called organic farming or agriculture. Organic farming is the agricultural management system that excludes the use of synthetic chemicals (fertilizers and pesticides) and employs such nutritional (crop rotation, biological nitrogen fixation, cover cropping, use of animal manures, non-synthetic and unprocessed minerals), plant protection (biopesticides) and cultural practices which not only improve crop productivity and produce quality but also ensure sustainability by enhancing soil quality and health [4, 5].

Management of fertility for optimum plant nutrition is big challenge in organic agriculture. Animal manures like cow manure (CM) and poultry manure (PM) are considered as important nutrient sources in organic farming. But there are some issues which reduce the efficiency of manures as good nutrient sources. Poultry manures have been reported to cause burning effects on crops due to its high nutrient and salt contents [6]. Similarly, poor nutrient use efficiency due to slow nutrient release which does not synchronize with plant need is another important issue [7, 8]. Prevailing scenario prompted us to devise some cost effective and sustainable approaches to enhance the effectiveness of manures. We thought that problem of burning effect of PM can be overcome by mixing it with cow manure. Similarly, after extensive review of literature we found that plant bio-stimulants like plant growth promoting rhizobacteria (PGPR) and humic acids (HA) can work as bio-activators to enhance the efficacy of manures due to their various promising features.

PGPR is a diverse group of free or associatively living soil bacteria that inhabit in the vicinity of plant roots and exert positive effects on plant growth, development and yield by the production of plant growth regulators, biological nitrogen fixation, solubilization of nutrients, mineralization of nutrient from organic matter through decomposition and enzymatic release and better nutrient uptake due to improved root growth [9-11]. Similarly, HA are components of humic substances (HS) that are end products of microbial decomposition and chemical degradation of dead biota [12]. HA can promote plant growth through improvement in root architecture and morphology, enhancement of nutrient availability, uptake and translocation, promotion of plant physiology and development due to their hormone-like activities and promotion of soil biological activity [13-18].

Keeping in view the characteristics of PGPR and HA we hypothesized that they can enhance the efficacy of manures through the activation of natural processes like nutrient release and uptake by various mechanisms described above. Extensive root system created by improved root growth and architecture by PGPR and HA can also enhance the effectiveness of manures by increasing the uptake of nutrients by exploring more and more soil volume. Stimulation of biological activity by HA can further enhance nutrient cycling through the action of microorganisms. Therefore to evaluate the potential of PGPR and HA for increasing effectiveness of manures we planned a field experiment using canola crop. In this experiment we used mixture of manures (CM and PM) prepared by blending the CM and PM in 3:1 to overcome the issue of burning effect of poultry manure.

II. MATERIALS AND METHODS

A. Experimental site and soil characteristic

The experiment was conducted at Agricultural Research Station of King Abdulaziz University, Hada Al-Sham (21o 48' 3" N, 39o 43' 25"E). Analysis of soil was carried out according to procedure given in Soil and Plant Analysis Laboratory Manual by Ryan and his colleagues [19]. According to results of analysis, soil was loamy sand in texture having pH (7.68), electrical conductivity (3.24 dS m^{-1}) and organic matter (0.65%). Nutrient analysis of soil revealed that it contains 0.054% total N, 6.4 mg kg^{-1} available P, 355 mg kg^{-1} extractable K and 54, 17, 2 and 5 mg kg^{-1} Fe, Zn, Mn and Cu respectively.

B. Collection of PGPR strain and seed inoculation

Pre-isolated strain of PGPR having plant growth promoting traits (IAA production, phosphate solubilization, ACC-deaminase activity and siderophore production) characterized by Ahmad and his co-workers [20] was used in this study. Broth culture of PGPR strain for seed inoculation was prepared in tryptic soy broth (TSB) medium according to Atlas [21]. Seed inoculation was done according to Baig and his co-workers [22]. First of all canola seeds were surface sterilized with 95% ethanol by dipping the seeds in ethanol for short time. After taking out the seeds from ethanol, they were washed with sterilized water and were immersed in 0.2% $HgCl_2$ solution for 3 minutes and washed again with sterilized water. Then surface sterilized seeds were inoculated with the broth mixed with 10% sugar solution, peat and clay (Kaolin) mixture (peat to clay ratio, 1:1 w/w). The seeds were shaken well until a fine coating appeared on seeds. Control seeds were treated with sterilized peat plus clay mixed with sterilized broth medium (without bacterial cells) and sugar solution. Inoculated seeds were placed overnight for drying under laboratory conditions at 26°C.

C. Field trial and treatment plan

To evaluate the combined effect of PGPR and HA for improving growth and yield of organically grown canola, a field trial was conducted according to split plot design in RCBD arrangement with four replications. Three levels of HA (HA_0= No HA, HA_{10}= 10 kg HA ha^{-1} and HA_{20}= 20 kg HA ha^{-1}) as main plot factor and two levels of PGPR (P_0= without PGPR and P_1= with PGPR) as sub-plot factor were used. First of all field was ploughed well with rotary plough. After that, 6 month old naturally composted cow manure CM) and poultry manure (PM) were obtained from Agricultural and Dairy Research Station of King Abdulaziz University, Hada Al-Sham. Then manures were mixed well in 3:1 (CM: PM) to get a fine blend. After getting a fine manure blend it was distributed and mixed well in the field @ 15 t ha^{-1} and main and sub plots were marked. Then different rates of humic acid were applied in the soil according to layout and mixed well in the soil. Finally, inoculated seeds were sown by keeping row to row and plant to plant distances of 40 cm and 30 cm respectively. Recommended cultural and agronomic practices were adopted for irrigation, weeding, plant protection and harvesting.

D. Data collection and statistical analysis

At the time of harvesting, data regarding growth and yield parameters such as plant height (cm), number of branches $plant^{-1}$, number of pods $plant^{-1}$, number of seeds pod^{-1}, 1000 seed weight (g) and seed yield (t ha^{-1}) were recorded to evaluate the effect of the treatments on crop growth and yield. All data was recorded according to standard procedures. Before harvesting, 10 random guarded plants in each sub-sub plot were tagged to record data regarding plant height (cm), number of branches $plant^{-1}$, number of pods plant-1 and number of seeds pod^{-1}. For seed yield an area of 1 m^2 in the center of each sub-sub plot (to avoid marginal effect) was harvested. Data recorded for each trait was separately exposed to the analysis of variance (ANOVA) to check significance among treatments using Statistix 8.1 computer software and means were then compared using least significant difference (LSD) test according to Steel and Torrie [23].

III. RESULTS

The analyzed data regarding the combined effect of HA and PGPR on growth and yield attributes of organically grown canola is given in Table 1. Results revealed that all the studied parameters were significantly improved by humic acid and PGPR application. However interaction of HA and PGPR was found significant only for 1000 seed weight and seed yield.

Mean comparison between different rates of HA (0, 10 and 20 kg HA ha^{-1}) for their effect on plant height showed that application of HA @ 20 kg ha^{-1} produced maximum plant height (102 cm) that was significantly ($p \leq 0.05$) higher compared to plots that received 0 and 10 kg HA ha^{-1}. Similarly, application of PGPR significantly induced more plant height (99 cm) than uninoculated treatment (96 cm). Number of branches per plant also showed similar trend. Maximum number of branches (26) was produced with 20 kg HA ha^{-1}, while 0 and 10 kg HA ha^{-1} produced 21 and 23 branches respectively. PGPR application produced more number of branches (25) than no PGPR application (22).

Number of pods $plant^{-1}$ showed somewhat different trend where 20 kg HA ha^{-1} with the production of 151 pods was statistically superior to both 0 and 10 kg HA ha^{-1}. Here, 10 kg HA ha^{-1} by producing 123 pods was also significantly higher than 0 kg HA ha^{-1} which produced 108 number of pods. Likewise, PGPR application also proved statistically better than no PGPR. PGPR application produced 134 pods while uninoculated control produced only 121 pods per plant. Regarding number seeds pod^{-1}, statistically higher numbers of seeds (27) were observed for 20 kg HA ha^{-1} followed by 22 and 19 seed pod^{-1} for 10 and 0 kg HA ha^{-1} respectively. PGPR inoculation also showed promising effects for improving number of seeds pod^{-1} and produced significantly higher (24) seeds pod^{-1} than the treatment without PGPR that produced 21 seeds pod^{-1}.

Data regarding the effect of HA and PGPR on 1000 seed weight of canola indicated that both humic acid and PGPR have interactive effect to improve this parameter. Interaction effect of PGPR and HA for 1000 seed weight presented in

Table 2 clearly indicated that maximum value of 1000 seed weight (2.45 g) was observed with 20 kg HA ha^{-1} at both levels of PGPR. Minimum value of 1000 seed weight (2.36 g) was recorded with the combination of 0 kg HA ha^{-1} and no PGPR application.

Table 1: Effect of humic acid and PGPR on growth and yield parameters of canola

Treatments	Plant Height (cm)	No. of branches $plant^{-1}$	No. of pods $plant^{-1}$	No. of seeds pod^{-1}	1000-seed weight (g)	Seed yield (t/ha)
Humic acid (kg/ha)						
HA_0 (0 kg)	94^b	21^b	108^c	19^b	2.38^b	1.54^c
HA_{10} (10 kg)	97^b	23^b	123^b	22^b	2.43^a	1.86^b
HA_{20} (20 kg)	102^a	26^a	151^a	27^a	2.45^a	2.40^a
LSD	4	2.33	2	3.9	0.04	0.11
PGPR						
P_0 (No PGPR)	96^b	22^b	121^b	21^b	2.41^b	1.70^b
P_1 (PGPR)	99^a	25^a	134^a	24^a	2.43^a	2.16^a
LSD	1.9	2.46	5	2.14	0.01	0.16
Significance						
Humic acid	**	**	**	**	**	**
PGPR	**	*	**	*	**	**
HA * PGPR	ns	ns	ns	ns	**	*

Means followed by the same letter (s) in each column and treatment showed no significant difference.
*, ** indicate significant differences at 0.05, 0.01 probability levels respectively while ns indicate non-significant difference.

Table 2: Interaction effect of PGPR and humic acid

Humic acid (HA)	PGPR (P)	1000-seed weight (g)	Seed Yield (t/ha)
HA_0	P_0	2.36^c	1.37^e
	P_1	2.40^b	1.70^d
HA_{10}	P_0	2.42^b	1.72^{cd}
	P_1	2.44^{ab}	2.00^{bc}
HA_{20}	P_0	2.45^a	2.02^b
	P_1	2.45^a	2.79^a
	LSD	0.02	0.28

As seed yield is closely related to yield components, so like yield components (pods $plant^{-1}$, number of seeds pod^{-1}, and 1000 seed weight) HA and PGPR application also showed significant effect on this parameter. For seed yield, significant ($p \leq 0.05$) interaction between HA and PGPR was observed (Table 2). The results presented in table clearly demonstrated that application of PGPR at each level of humic acid has produced more seed yield than without PGPR at the same level of humic acid. Maximum seed yield (2.79 t ha^{-1}) was achieved with the combined application of PGPR and HA @ 20 kg ha^{-1}, which is significantly higher than all other interactions. Minimum seed yield (1.37 t ha^{-1}) was observed where no PGPR and HA was applied.

IV. Discussion

It was confirmed from the results that combined application of PGPR and HA significantly improved growth and yield attributes of canola under field conditions. Previously, PGPR have been reported to enhance plant growth and yield by the production of PGRs (IAA, gibberellins, and cytokinins etc.) and improved nutrient availability and uptake by nutrient solubilization, nutrient cycling and enhanced root growth [24, 25, 9]. Possession of plant growth promoting activities by the strain used in this study already confirmed by Ahmad and his co-workers [20] further strengthens our results.

The impact of HA growth and yield parameters may reasoned to its hormone-like activity and role in plant metabolism [13, 26]. Auxin-like activity of HA has also been reported earlier by some researchers [27, 16]. It is an established fact that PGRs accelerate the vital developmental processes of cell elongation, cell division which cause improvement in plant growth [28]. Moreover, both HA and PGPR have been reported to indirectly promote plant growth parameters by enhancing nutrient availability and uptake especially N which has significant role in the promotion of vegetative growth [29].

Different researchers have reported the effects of HA and PGPRs on various physiological and metabolic processes, nutrients uptake and translocation and accumulation of photosynthates. For example, El-Nemr and his co-workers [30] conducted a field trial on cucumber in Egypt for two seasons and noted that humic acid significantly increased number of flowers and fruit per plant, fruit set, mean fruit weight, fruit length and diameter, and yield per plant. Likewise, Yildrim [31] observed improvement in growth and yield parameters of tomato by the accumulation of total soluble solids and ascorbic acid contents with humic acid application. Similarly, Karakurt and his co-workers [32] recorded enhancement in total soluble sugars, reducing sugars, and chlorophyll b content of pepper with parallel improvement in growth and yield by humic acid application. Findings of our work regarding improvement in growth and yield parameters by PGPR are also in line with other researchers [33-35] who have reported improvement in growth and yield of crops by PGPR.

V. CONCLUSION

The results of this study are very promising and inspiring. The improvement of canola growth and yield by the combined application of PGPR and in this region is of great value. Because, canola can be successfully grown in dry land or under irrigation even with saline soil conditions. Application of this approach at large scale can be helpful to boost canola production to overcome the problem of fodder shortage.

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