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Full Length Article

Evaluating Mungbean Performance under Different Types and Rates of Humic Acid Application in Arid Conditions of Saudi Arabia

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Abstract

Humic acid application has considerable effect on crop productivity in sandy soils due to its unique biochemical characteristics. This study was conducted to optimize the mungbean production in arid conditions of Saudi Arabia by tracing out most suitable combination of levels and types of humic acid (HA) application. In this two-year study, mungbean cultivar 'NM-2006'collected from NIAB, Pakistan, was grown with HA application in liquid and solid forms at the rates of 0, 20, 40 and 60 kg ha^{-1}. Both types and levels of HA application had significant effect on growth, gas exchange traits, photosynthetic pigments, yield and quality of mungbean while the interactive effect of types and rates of HA remained non-significant for all traits in both years. Among types, solid form of HA significantly improved plant height, leaf area index, root and shoot dry weights, gas exchange traits, photosynthetic pigments, yield and related traits, and grain quality of mungbean compared with its liquid form. Likewise, higher levels of HA, 60 kg ha^{-1} was the best, gradually improved all above stated traits of mungbean in both years. Similarly, SEM based evaluation revealed notable changes in the distribution of leaves mineral content; however, this change was maximum for solid HA application particularly at 60 kg ha^{-1}. In conclusion, HA application at 60 kg ha^{-1} in solid form seemed a viable option to improve growth, gas exchange traits, photosynthetic pigments, and grain yield and quality of mungbean in sandy soils of arid climate of Saudi Arabia. © 2020 Friends Science Publishers

Keywords: Photosynthesis; Mineral nutrients; Agronomic traits; Humic acid; Grain yield; Grain quality

Introduction

Mungbean (*Vigna radiata* L.) is an important grain legume crop. It is a short duration crop which occupies 7 million hectares, mainly in Asia and rapidly spreading across the globe, owing to its high protein, fiber, antioxidants and phytonutrients (Itoh *et al.* 2006; Nair *et al.* 2019). This crop is highly suitable for arid regions of Saudi Arabia, as it can tolerate drought stress and don't need large amount of irrigation water due to its well-developed tap and lateral root system, which facilitates in rapid water absorption (Ahmed *et al.* 2015).

Sustainability of agriculture not only needs effective mineral fertilization containing macro and micronutrients, but also plant bio-stimulants having biologically active compounds (Calvo *et al.* 2014; Du-Jardin 2015). The prime function of these bio-stimulants is to accelerate natural processes stimulating the nutrient uptake and assimilation (Halpern *et al.* 2015; Yakhin *et al.* 2017). Humic substances impacting plant growth both directly and indirectly are formed by microorganisms as a result of chemical and biological humification of animal and plant matter (Shah *et al.* 2018). Indirectly humic substances improve soil microbiota, soil structure and soil chemistry, while directly these affect photosynthesis, respiration rate, enzymatic activities, protein formation and carbohydrates assimilation (Dawood *et al.* 2019). Humic acid (HA) is an important component of humic substances that improves the soil water holding capacity (Khaled and Fawy 2011). Being a bio-stimulant, HA regulates enzymatic activity that triggers various plant physiological processes leading toward an increase in yield components (Waqas *et al.* 2014). HA is an important and integral part of soil organic matter which is not only a plant bio-stimulant, instead it is a soil conditioner that improves soil texture, structure and physiochemical features (Calvo *et al.* 2014).

Saudi Arabia comprises vast arid regions with sandy soils having poor water and nutrient holding capacity, therefore the use of HA is logically recommended for arable crops cultivation. However, prevailing arid climatic conditions with vast sandy soils poses serious challenges to sustainable agricultural activities. Among legumes, mungbean has been targeted due to its importance in Saudi lifestyle and can be grown on marginal soils with some

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success. Therefore, this study was designed to optimize the types and rates of HA application to improve mungbean growth, photosynthesis, and grain yield and quality. It was hypothesized that being a good soil conditioner, HA application has the potential to improve mungbean growth and yield on sandy soil in arid climatic conditions of Saudi Arabia.

Materials and Methods

Seeds of mungbean variety 'NM- 2006'were collected from NIAB (Nuclear Institute of Agriculture and Biology), Faisalabad, Pakistan and used as experimental material. This two-year study was conducted to assess the effectiveness of HA types and rates on agronomical, physiological, yield and quality parameters of mungbean. Field experiments were conducted at King Abdulaziz University, Jeddah, Saudi Arabia in two consecutive years 2016–2017 and 2017–2018.

Experimental details

Mungbean was sown with HA application in both solid and liquid form at four different rates (0, 20, 40 and 60 kg ha^{-1}). HA in solid phase was spread and manually incorporated to the top 20 cm layer of soil before cultivation during each growing season. However, the liquid phase of HA was equally divided into three doses that afterward applied at the rates of 0, 20, 40 and 60 kg ha^{-1}. After complete dissolution in water, each dose was manually sprayed on soil surface. First dose was applied after complete germination followed by second dose two weeks after, while third dose was applied at the start of flowering. Experiment was laid out following randomized complete block design (RCBD) under split plot arrangement keeping HA types in main plots and rates in sub-plots, respectively. Experiment was repeated four times with net plot size of 2 m × 3 m.

Crop husbandry

Prior to cultivation, soil was prepared by two ploughing followed by cross ploughing and laddering. Afterward, 33–40 seeds per meter square were sown using drill at the depth of 3–4 cm in well leveled, moist and weed free soil in 25 cm spaced rows with plant to plant distance of 10 cm. To keep the soil moist, the drip irrigation system was used on daily bases for all stages of plant. Crop was fertilized at the rate of 40 kg each of nitrogen, phosphorus and potassium ha^{-1} using of NPK (20-20-20) fertilizer as source. Fertilizer was applied in three splits at 20, 40 and 60 days after sowing as fertigation (Akhtar *et al*. 2017). In order to remove weeds, manual weeding was done after every 20 days.

Data collection

Growth parameters like plant height, leaf area index, and shoot and root dry weights were measured following Akhtar *et al.* (2017). Average height of 10 randomly selected plants from each sub-plot was measured with meter rod before harvesting and averaged. Leaf area index was measured at each phenological stage using Plant Canopy Analyzer. Average root and shoot dry weights of completely dried ten randomly selected plants were calculated using digital electric balance. Physiological parameters, for instance stomatal conductance, transpiration rate and photosynthesis rate were measured during morning time before 10 a.m., from the abaxial surface of fully expanded leaves by using Syrus 3 upgraded model SC-1, 2011 (Decagon Devices, 2011). Total chlorophyll and carotenoids were determined by using the protocol of Mahmood *et al.* (2016).

Above mentioned randomly selected ten plants were used to record average number of branches and pods per plant, and pod length. For grain and biological yield, two central rows were harvested and kept in field for three days for drying, tied into bundles and weighed using spring balance to record biological yield. After that pods were threshed manually, grains were separated and weighed using electrical balance to record grain yield.

For seed quality parameters, automatic Kjeldahl Analyzer was used to measure grain nitrogen contents, spectrophotometer at 410 nm was used to record grain phosphorus contents while flame photometer was used to estimate grain potassium contents. Moreover, total protein and carbohydrates content were recorded following the protocol of Madar *et al.* (2017).

Scanning electron microscopy

The SEM micrography of leaves from selected plant samples was done with objective to compare the effect of HA types and rates on the uptake of nutrient elements. Energy dispersive X rays (EDX) system was used within the microscope due to its high resolution, fast counting and analytical tendency. Moreover, this technique is beneficial owing to its tendency to characterize the sample without prior need of preparation. The leaves samples from mungbean were placed inside the detectors of EDX system under X-rays photons. The data obtained after EDX analysis consists of SEM spectrographs whose peaks indicate the assimilated elements within leaf samples. In EDX generated spectrograph x-axis indicates the energy, while y-axis represents the number of X-rays counts. The positions of peaks in spectrograph facilitated the identification of elemental, while their heights helped to quantify the nutrient elements inside leaves. Moreover, SEM was already installed with features of auto-identification and quantification of the elemental peaks.

Statistical analysis

To check the significance of applied treatments, data attained for all traits were subjected to two-way analysis of variance (ANOVA) using computer-based software statistics 8 (v. 8.1, © 1985–2005). In case of significant effects, means were compared using least significant difference (LSD) test at 5% level of probability (Steel *et al.* 1997).

Results

Growth parameters

Both HA types and rates significantly improved the growth of mungbean while their interactive effect was non-significant in both years of study (Table 1). Solid form of HA significantly improved plant height, LAI, shoot fresh and dry weight, and root fresh and dry weight of mungbean compared with its liquid form in both years of study (Table 1). Likewise, with increasing HA rates, the highest improvement in growth parameters was noticed at 60 kg ha^{-1} in both years of study (Table 1).

Physiological traits

Both HA types and rates significantly improved the values of physiological parameters of mungbean while their interaction depicted non-significant effect during both years (Table 2). Solid form of HA significantly improved photosynthesis rate, stomatal conductance, transpiration rate, total chlorophyll and carotenoids of mungbean compared with its liquid form in both years of study (Table 2). Likewise, with increasing HA rates, maximum rate *i.e.*, 60 kg ha^{-1} showed maximum improvement in physiological parameters during both years of study (Table 2).

Yield and related traits

Both HA types and rates significantly affected the yield and related traits of mungbean while their interactive effect was non-significant in both years of study (Table 3). Solid form of HA significantly increased branches plant^{-1}, pods plant^{-1}, pod length, 100-seed weight, seed yield and biomass yield of mungbean compared with its liquid form in both years of study (Table 3). Moreover, with increasing HA rates, maximum rate *i.e.*, 60 kg ha^{-1} showed maximum improvement in yield component in both years of study (Table 3).

Seed quality parameters

In both years of study HA types and rates significantly improved the grain P and K contents while had non-significant effect on grain N, carbohydrates and protein contents of mungbean. Besides the interactive effect of HA types and rates remained non-significant (Table 4). Solid form of HA significantly enhanced P and K content of mungbean compared with its liquid form in both years of study (Table 4). Moreover, with increasing HA rates, maximum rate *i.e.*, 60 kg ha^{-1} showed maximum improvement in seed quality parameters in both years of study (Table 4).

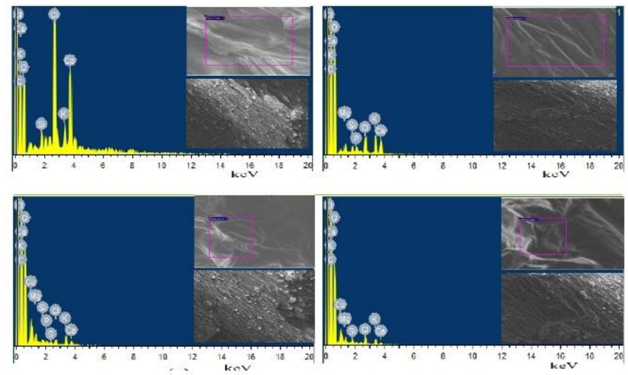

Fig. 1: SEM micrographs demonstrating the difference in elemental distribution in the leaves of mungbean under different types and rates of humic acid

a. Distribution of elements under solid HA at the rate of 60 kg ha^{-1}
b. Distribution of elements under liquid HA at the rate of 60 kg ha^{-1}
c. Distribution of elements under solid HA at the rate of 40 kg ha^{-1}
d. Distribution of elements under liquid HA at the rate of 40 kg ha^{-1}

SEM micrographs

SEM micrographs of randomly selected best performing mungbean plants for both types of HA at rate 40 and 60 kg ha^{-1} were generated (Fig. 1). They showed visible variation in the assimilation and distribution of nutrient elements in the leaves of mungbean at varying rates of both types of HA. Moreover, the SEM micrographs of targeted area revealed the highest uptake of mineral elements for solid HA at 60 kg ha^{-1} concentration as compared to concentration and type as indicated by the broader peak area of Figure 1a. Besides, variation in peak positions of minerals was noticed in micrographs for different types of HA at different rates, which reflect varying impact of HA types and rates on the distribution of minerals in leaves (Fig. 1a–d).

Discussion

Results of this two-year field study unveiled that HA application, solid type specially, improved growth, gas exchange traits, yield related traits and grain quality of mungbean on sandy soil under arid climatic conditions of Saudi Arabia (Table 1–4). Higher grain yield of mungbean at higher rate *i.e.*, 60 kg ha^{-1} of HA application was due to notable expansion in entire yield related traits. Moreover, significant expansion in yield-related traits was primarily due to substantial improvement in photosynthesis owing to higher stomatal conductance and synthesis of photosynthetic pigments (Shafeek *et al.* 2013; Tables 1–3). In another recent study, Akhtar *et al.* (2017) also concluded higher mungbean yield with HA application of 60 kg ha^{-1} in both solid and liquid forms due to significant elevation in yield-related traits like number of pods and grains plant^{-1}, and grain size.

Humic substances improve plant growth both directly and indirectly as a result of chemical and biological

Table 1: Effect of humic acid types and rates of application on growth of mungbean

Treatments	Plant height (cm)		Leaf area index		Shoot dry weight (g plant^{-1})		Root dry weight (g plant^{-1})	
	2016–2017	2017–2018	2016–2017	2017–2018	2016–2017	2017–2018	2016–2017	2017–2018
HA types								
Solid	36.63 a	43.7 a	2.58 a	2.67 a	21.2a	26.7a	2.26 a	2.58 a
Liquid	32.75 b	38.51 b	2.20 b	2.28 b	16.6 b	23.0 b	2.16 b	2.44 b
LSD value at 0.05	1.37	1.70	0.04	0.04	0.37	0.21	0.08	0.11
HA rates (kg ha^{-1})								
0	30.78 b	38.3 b	1.85 d	1.90 d	14.3 d	17.7 d	1.90 d	2.15 d
20	31.52 b	39.5 b	2.04 c	2.11 c	17.5 c	22.1 c	2.07 c	2.35 c
40	34.32 a	42.7 a	2.72 b	2.80 b	24.1 b	27.6 b	2.33 b	2.66 b
60	34.28 a	42.9 a	2.80 a	2.89 a	22.0 a	30.4 a	2.43 a	2.80 a
LSD value at 0.05	1.17	1.48	0.03	0.01	1.05	0.25	0.08	0.11
Significance								
HA (Types)	**	**	**	**	**	**	**	**
HA (Rates)	**	**	**	**	**	**	*	**
Types × Rates	ns	ns	ns	ns	ns	ns	ns	ns

Means following the same letters, within a column for each trait, are not statistically different from each other at $P \leq 0.05$
HA=Humic Acid; **= Significant at $P \leq 0.01$; ns= Non-Significant

Table 2: Effect of humic acid types and rates of application on physiological parameters of mungbean

Treatments	Photosynthesis Rate (μmm^{-2} S^{-1})		Stomatal Conductance (mmm^{-2} S^{-1})		Transpiration Rate (mmm^{-2} S^{-1})		Total Chlorophyll (g kg^{-1})		Carotenoids (mg kg^{-1})	
	2016–2017	2017–2018	2016–2017	2017–2018	2016–2017	2017–2018	2016–2017	2017–2018	2016–2017	2017–2018
HA types										
Solid	28.54 a	35.82 a	885.60 a	940.95 a	12.98 a	16.50 a	1.60 a	1.68 a	3.00 a	3.38 a
Liquid	23.63 b	29.62 b	790.90 b	843.52 b	12.001 b	15.34 b	0.87 b	0.95 b	2.15 b	2.05 b
LSD value at 0.05	0.32	0.40	7.88	8.25	0.05	0.07	0.06	0.056	0.05	0.05
HA rates (kg ha^{-1})										
0	18.05 d	22.61 d	772.55 d	821.81 d	11.18 d	13.19 d	1.25 d	1.34 c	2.65 d	2.85 d
20	23.05 c	28.90 c	812.17 c	863.70 c	12.91 c	15.22 c	1.50 c	1.52 b	2.85 c	3.00 c
40	30.70 b	38.60 b	879.48 b	935.60 b	14.54 b	17.23 b	1.56 b	1.58 b	3.01 b	3.10 b
60	32.45 a	40.78 a	891.0 a	947.85 a	15.32 a	18.13 a	1.66 a	1.69 a	3.15 a	3.25 a
LSD value at 0.05	1.30	0.32	3.46	3.70	0.13	0.13	0.05	0.06	0.045	0.05
Significance										
HA types	**	**	**	**	**	**	**	**	**	**
HA rates	**	**	**	**	**	**	**	**	**	**
Types × Rates	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Means following the same letters, within a column for each trait, are not statistically different from each other at $P \leq 0.05$
HA=Humic Acid; **= Significant at $P \leq 0.01$; ns= Non-Significant

Table 3: Effect of humic acid types and rates of application on yield components of mungbean

Treatments	Branches plant^{-1}		Pods plant^{-1}		Pod length (cm)		100-seed weight (g)		Seed yield (t ha^{-1})		Biomass yield (t ha^{-1})	
	2016–2017	2017–2018	2016–2017	2017–2018	2016–2017	2017–2018	2016–2017	2017–2018	2016–2017	2017–2018	2016–2017	2017–2018
HA types												
Solid	20.59 a	23.68 a	23.85 a	28.28 a	15.25 a	16.28 a	1.48 a	1.69 a	1.07 a	1.27 a	6.54 a	7.45 a
Liquid	17.73 b	20.40 b	19.40 b	22.95 b	13.01 b	13.90 b	1.38 b	1.60 b	0.92 b	1.08 b	5.52 b	6.35 b
LSD value at 0.05	0.40	0.52	0.80	0.83	0.23	0.25	0.007	0.006	0.02	0.03	0.05	0.06
HA rates (kg ha^{-1})												
0	12.34 d	16.92 d	16.45 d	19.48 d	10.10 d	9.75 d	1.33 d	1.55 d	0.91 c	1.05 c	5.07 d	5.81 d
20	17.35 c	19.93 c	19.85 c	23.52 c	11.65 c	12.46 c	1.38 c	1.60 c	0.97 b	1.13 b	5.41 c	6.22 c
40	21.87 b	25.20 b	24.45 b	28.95 b	16.81 b	17.98 b	1.44 b	1.65 b	1.04 a	1.24 a	6.65 b	7.61 b
60	22.69 a	27.50 a	25.68 a	30.49 a	17.89 a	19.18 a	1.48 a	1.73 a	1.05 a	1.25 a	6.88 a	7.86 a
LSD value at 0.05	0.26	0.30	0.28	0.35	0.33	0.34	0.002	0.004	0.03	0.02	0.05	0.04
Significance												
HA types	**	**	*	*	*	**	**	**	**	**	**	**
HA rates	**	**	**	**	**	**	**	**	**	**	**	**
Types × Rates	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Means following the same letters, within a column for each trait, are not statistically different from each other at $P \leq 0.05$
HA=Humic Acid; *= Significant at $P \leq 0.05$; **= Significant at $P \leq 0.01$; ns= Non-Significant

humification of animal and plant matter (Shah *et al*. 2018). Indirectly humic substances improve soil microbiota, soil structure and soil chemistry; while directly these affect photosynthesis, respiration rate, enzymatic activities, and carbohydrates assimilation (Dawood *et al*. 2019). Moreover, HA is an important and integral part of soil organic matter which is not only a plant bio-stimulant, instead it is a soil conditioner that improves soil texture, structure, water holding capacity and physiochemical features (Khaled and Fawy 2011; Calvo *et al.* 2014). Due to these features, HA

Table 4: Effect of humic acid types and rates of application on seed quality parameters of mungbean

Treatments	Grain nitrogen (%)		Grain phosphorus (%)		Grain potassium (%)		Grain protein (%)		Grain carbohydrates (%)	
	2016–2017	2017–2018	2016–2017	2017–2018	2016–2017	2017–2018	2016–2017	2017–2018	2016–2017	2017–2018
HA types										
Solid	4.34 a	4.55 a	0.38 a	0.46 a	1.55 a	1.69 a	28.19	28.11	59.61	58.75
Liquid	4.34 a	4.55 a	0.30 b	0.36 b	1.36 b	1.50 b	28.06	28.64	59.00	58.24
LSD value at 0.05	ns	ns	0.004	0.02	0.02	0.03	ns	ns	ns	ns
HA rates (kg ha^{-1})										
0	3.64 d	3.83 d	0.25 d	0.26 d	1.21 d	1.33 d	21.75 d	22.23 d	58.00 d	56.75 d
20	4.11 c	4.34 c	0.29 c	0.33 c	1.39 c	1.512 c	26.73 c	25.13 c	58.75 c	57.75 c
40	4.72 b	5.00 b	0.37 b	0.43 b	1.60 b	1.75 b	30.54 b	32.44 b	60.25 b	61.35 b
60	4.95 a	5.55 a	0.40 a	0.49 a	1.65 a	1.79 a	31.55 a	34.65 a	62.50 a	56.50 a
LSD value at 0.05	0.005	0.02	0.003	0.03	0.04	0.01	0.07	0.05	0.65	0.70
Significance										
HA types	ns	ns	**	**	**	**	ns	ns	ns	ns
HA rates	**	**	**	**	**	**	**	**	**	**
Types × Rates	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Means following the same letters, within a column for each trait, are not statistically different from each other at $P \leq 0.05$
HA=Humic Acid; **= Significant at $P \leq 0.01$; ns= Non-Significant

application improved water and nutrient uptake which in turn resulted in higher LAI, stomatal conductance and higher synthesis of chlorophyll and carotenoid contents. Nonetheless, SEM analysis also highlighted more nutrients uptake at higher rate (60 kg ha^{-1}) of HA application (Fig. 1). Similarly, Trevisan *et al*. (2011) reviewed the positive impact of humic substances on uptake of nutrient elements; for example, nitrogen, phosphorus, potassium, Sulpher and magnesium *etc*. and concluded that at varying rates different methods of HA differently affect their uptake. Mora *et al*. (2010) reported positive effects of HA on nutrient uptake and gas exchange traits in cucumber. Generally, HA adheres tightly to cell wall and absorbed by plant roots from where it is partly transferred to shoots where it affects plant metabolism directly (Nardi *et al*. 2002).

The well-developed plant canopy (LAI) and photosynthetic pigments captured more solar radiation, and higher stomatal conductance enhanced carbon influx leading to higher photosynthesis (Ameri and Tehranifar 2012; Tables 1–3). Therefore, more accumulation of photo-assimilates resulted in significant expansion in yield related traits leading to higher mungbean yield in this study. In another study Waqas *et al.* (2014) reported substantial improvement in mungbean yield at higher rates of HA application due to more availability of nutrients from soil to plants. These findings are consistent with the results obtained by Bakry *et al*. (2013) in flax seed and El-Bassiouny *et al*. (2014) in wheat. Correspondingly, HA types and rates significantly improved seed quality characteristics of mungbean such as higher grain nitrogen, phosphorus, potassium contents, and protein and carbohydrates (Table 4). The significant improvement in grain mineral contents owing to HA application was chiefly linked with more macro- and micronutrients uptake (Fig. 1; Trevisan *et al.* 2011). Moreover, better growth and physiological performance enabled plants to assimilate more photosynthates which lead to higher grain protein and carbohydrate contents (Delfine *et al.* 2005; Ozlem *et al.* 2017).

Results of this study also unveiled that solid form of HA application was more beneficial in improving growth, gas exchange traits, and grain yield and quality of mungbean (Tables 1–4). The possible reason behind was high staying and adherence tendency of solid form of HA, which facilitated more uptake of micro and macro-nutrients (Fig. 1; Shah *et al*. 2018). This improvement was more dynamic for the increasing rates of solid HA as compared to liquid because sandy soils have poor holding tendency for liquid form of HA (Afzal *et al*. 2017; Khaled and Fawy 2011). Therefore, the application of HA in solid form seemed more appropriate choice than liquid form on sandy soils of Saudi Arabia. Earlier Waqas *et al.* (2014) and Akhtar *et al.* (2017) also concluded that solid form of HA is more suitable than its liquid form to improve mungbean growth and productivity. The possible reason could be the high affinity of solid HA for sandy soil that facilitates efficient nutrients and water uptake (Khaled and Fawy 2011; Shah *et al*. 2018).

Conclusion

Humic acid application, solid form in particular, improved mungbean productivity in both years of study. Therefore, mungbean can be effectively cultivated in prevailing conditions of Kingdom of Saudi Arabia using humic acid as supplement particularly in solid form at the rate of 60 kg ha^{-1}. The outcomes of this study provided some future guidelines for the optimization and adaptability of testing of exotic legume crops in arid conditions of Saudi Arabia.

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