Journal Homepage: -www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI:10.21474/IJAR01/13408
DOI URL: http://dx.doi.org/10.21474/IJAR01/13408

RESEARCH ARTICLE

USES OF BIOFERTILIZATION TO PRODUCE TROPICAL GRASS FORAGE IN QUINTANA ROO MÉXICO

Sosa-Rubio Edgar Enrique, Herrera-Cool Gilbert José and Zavaleta-Córdova María Del Carmen
Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP), Campo Experimental Chetumal, Km 3.5 carretera Chetumal - Bacalar, Chetumal, Quintana Roo, México.

Manuscript Info

Manuscript History
Received: 14 July 2021
Final Accepted: 18 August 2021
Published: September 2021

Key words:-
Strains, Fungi, Bacteria, Guinea, Mombaza

Abstract

The objective of this study was to evaluate the biofertilizers effect in *Panicum maximaum* (cv. Mombaza) and *Brachiaria brizantha* tropical grasses production. Microorganisms were obtained in rhizosphere of plants. To establish an effective symbiosis with native strains of *Azospirillum*, *Azotobacter* and mycorrhizal fungi, experiments were carried out in greenhouse and field. The biofertilizers used in greenhouse were combined (CC), semisolid medium Nitrogen free with malate as nitrogen source (NFB), Azotobacter (azot) and Azospirillum (Azos). For mycorrhizal fungi, 6 treatments were used: T1-control, T2-fertilized, T3-brown spore, T4-honey spore, T4-black spore and T5-commercial spore. The microorganism used in field were those that showed effectivity in greenhouse. The treatments in field were T1: control, T2: inorganic fertilizer, T3: *Azospirillum* + *Azotobacter*, T4: mycorrhizal and T5: commercial biofertilizer. The variables evaluated were dry weight (DW), radicular weight (RW), radicular volume (RV), stem diameter (SD) and total height (TH). Results for *B. brizantha* indicate differences ($P \leq 0.05$). Application of *Azospirillum* + *Azotobacter* (T3) favored the development of the height of the plant and the diameter of the stem. The commercial biofertilizer (T5) increased the production of dry matter with 0.99 kg/m^2. In respect with *P. maximum* (cv. Mombaza) grass, they were not detected significative differences ($P \geq 0.05$) between treatments, however, the biological results showed that inorganic fertilizer (T2) increased the dry matter production with 1.34 kg / m^2 in comparison with *Azospirillum* + *Azotobacter* (T3) that showed 0.72 kg / m2.

Introduction:-

In Mexico exits many tropical forage plants that are used by farmers in livestock (Jose and Dollinger, 2019), those forages species contain high nutritive value that can be compared with European species (Muciño-Álvarez *et al*., 2021). Those species can permit the creation of annual or semi-perennials crops, as well as artificial long-lasting forage lands (Williams *et al*., 2017; Legge *et al*., 2019). The species that are usually used by many farmers are the grasses, this is because of the energy source that provides to domestic animals and wild fauna (Keesing*et al*., 2018). Grasses as well as other forage species need to be fertilized to generate production of high nutritive quality (Kılıçalp *et al*., 2018). However, to obtain high quality forage production is indispensable the use of inorganic fertilizers. In

Corresponding Author:- Sosa-Rubio Edgar Enrique
Address:- Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP), Campo Experimental Chetumal, Km 3.5 carretera Chetumal - Bacalar, Chetumal, Quintana Roo, México.

recent years, has been demonstrated that this practice is harmful to environment and soil, furthermore that inorganic fertilizers are expensive (Koli *et al*., 2019; Baweja *et al*., 2020; Thakur *et al*., 2021). One of the alternatives to reduce the use of inorganic fertilizers are the application of promoter microorganisms of plant growing. These microorganisms are usually called biostimulants, biofertilizers or inoculants (Mącik *et al*., 2020). Biofertilizers are microorganisms who live associated with root plants and provide nutritional elements indispensable for growing and plant production (Aguirre-Medina, 2006). The biofertilizer most used are the *Rhizobium* and *Azospirillum* generous bacteria, as well as *Glomus* generous mycorrhizal fungi, this last usually come from other regions and is difficult their acquisition by farmers (García *et al.*, 2006). The principal function of some bacteria is to assimilate the nitrogen for plants (Etminani and Harighi, 2018;Pajčin *et al*., 2021). On the other hand, mycorrhizal fungi associated with plants can transport phosphorous (P) through mycelium (Etminani and Harighi, 2018; Raklami *et al*., 2021). Furthermore, it can protect to plants against harmful microorganisms and can increase the life of root hair and provide tolerance to heavy metals and toxic substances (Hashem *et al*., 2018; Zhang*et al*., 2018; García-Sánchez *et al*., 2021). It exists other type of microorganisms as *Azospirillum* which provide plant growth regulators (PGRs) to soil around the root (Chávez-Herrera *et al*., 2018; Coniglio *et al*., 2019). These PGRs induce root ramification that implies an efficient obtention and absorption of nutrients (Kashyap *et al*., 2017). Those microorganisms play an important role in forage and grass production due to the biological balance that provide and for being a friendly option with the environment (Modesto *et al*., 2018; Kour *et al*., 2020). Other advantages of biofertilizers is that can be produce with natural products, is not polluting and it does not require hydrocarbons for elaboration. This could be considered an economic method that can contribute in farming production. Because of the above mentioned, the aim of this study was to evaluate the effect of biofertilizers in the production of the tropical grasses in Quintana Roo, Mexico.

Materials and Methods:-

Study área

The present study was carried out in Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP), campo experimental Chetumal, located in Othon P. Blanco, Quintana Roo at 3.5 km of Xul-Ha community with 21°30' N and 89°29' W coordinates at 10 masl. Climate conditions are 27.6° C and 62.3% of relative humidity on average, annual medium precipitation is 1300 mm and the period with the most precipitation was from June to November with 70% of precipitation.

Isolation of Azospirilum and Azotobacter rhizobacteria

Isolations of *Azospirillum* and *Azotobacter* were carried out from rhizosphere of *B. brizantha* and *P. maximum* (cv. Mombaza) grasses. These grasses were cultured in luvisol and cambisol soils. For the first step, 5 plants free of plagues and diseases were randomly selected. Afterwards, five samples of 500 g of soil per plant were collected in rhizosphere zone. Samples were collected with Hoffer barrier and deposited in nylon bags for analyzing in laboratory. The 25 samples per type of soil were homogenized before analysis. The technique used for isolation was serial dilution (CIAT, 1988). 5 *Azotobacter* and 4 *Azospirillum* strains were isolated and stored in refrigerator at 8°C for using subsequently in biofertilizer production. Finally, the strains were purified by streak plate method. the strains were cultured in five petri dishes per chilled strain.

Isolation of mycorrhizal fungi

Spores were obtained from soil rhizosphere of *P. atratum, B. brizantha* and *B. humidícola* species. For isolation, 200 g of soil per each grass was weighted. Then, soil was mixed and shaken per five minutes in 2000 mL of distillated water. Afterwards, the mixture was settled for three minutes. Posteriorly, mixture was sieved in mesh sieves of 03800 mm, 00340 mm and .044 mm to obtain the spores.

Biofertilizers preparation

For biofertilizer preparation, it was prepared suspensions to inoculate directly the plant. *Azotobacter* was multiplied in charcoal medium combined and *Azospirullum* in Nitrogen Free Malatum (NFB) medium. The strains obtained were 5 for *Azotobacter* and 4 for *Azopirillum*. On the other hand, mycorrhizal was cultured in tramp plant and then collect the air parts with inoculant. Air parts were dried in temperature room to obtain the microbial inoculum. The microbial inoculum was crushed in a hammer mill and put inside of plastic bags for conservation. Plastic bags with inoculum were conserved in 4°C. For subsequently application, the suspension was prepared dissolving 1g per liter (w/v) and sprinkled on stablished plants in Leonard jars.

Biofertilizers test in controlled conditions

Essays in greenhouse were carried out to identify the behavior and development of selected strains of *Azospirillum*, *Azotobacter* and mycorrhizal fungi. These microorganisms were applied in grass to evaluate the effectivity. 36 pots with three replicates per treatment were evaluated in a random completely design. Inoculation was applied in two forage grass species. The biofertilizers used were combined (CC), semisolid medium Nitrogen free with malate as nitrogen source (NFB), *Azotobacter* (azot) and *Azospirillum* (Azos). The treatments evaluated were the T1: control, T2: fertilized species, T3: CC-azot1, T4: CC-azot2, T5-CC-azot3, T6-CC-azot4, T7-CC-azot5. T8-NFB-azos6, T9-NFB-azos7, T10-NFB-azos8 and T11-NFB-azos9. The variables evaluated were total height, dry weight and leaf length.

Biofertilizers test in field

The experiment used in field was random completely block design. The experimental unities were homogenous. In this experiment were used the *B. brizantha* and *P. maximum* (cv. mombaza) grass species. It was evaluated 5 treatments with three replicates. The microorganism used in this experiment were those that showed effectivity in greenhouse. Treatments were T1: control, T2: inorganic fertilizer, T3: *Azospirillum* + *Azotobacter*, T4: mycorrhizal and T5: commercial biofertilizer. The useful plot evaluated was $2m^2$ and sample plot was $1m^2$. The evaluation was carried out each month to measure the dry weight, radicular weight, radicular volume, stem diameter and total height.

Results:-

Microorganisms obtained in two soil types

Results indicated the presence of Azospirillum and Azotobacter both luvisol and cambisol soils. This presence was observed in natural way around rhizosphere grasses. Furthermore, the most abundant microorganism was Azotobacter contrary to Azospirillum (Table 1).

Table 1:- Colony-forming units observed in *Azospirillum* and *Azotobacter* per grams in luvisol and cambisol soils.

Dilutions	*Azospirillum* (CFU/g)			*Azotobacter* (CFU/g)	
	Vertisols	Cambisols		Vertisols	Cambisols
10^4	11.2 x 10^4	14.1 x 10^4		1.37 x 10^4	9.70 x 10^4
10^5	2.53 x 10^5	2.40 x 10^5		23.2 x 10^5	15.8 x 10^5
10^6	1.90 x10^6	3.80 x10^6		25.0 x10^6	12.3 x10^6

Essays evaluation in greenhouse

T7 and T4 treatments in greenhouse with bacteria inoculation of *B. brizantha* showed significative differences ($P \leq 0.05$) in total height of the plant with 55 and 36 cm respectively. The plant evaluated with synthetic fertilizer (T2) showed 11 cm in total height of the plant (Figure 1). The mycorrhizal fungi inoculation increased the total height and production of dry matter for *B. brizantha*. The combined with *Azotobacter3* (T5) was one of the best treatments ($P \leq 0.05$) producing 9.9 g of dry matter (Figure 1b).

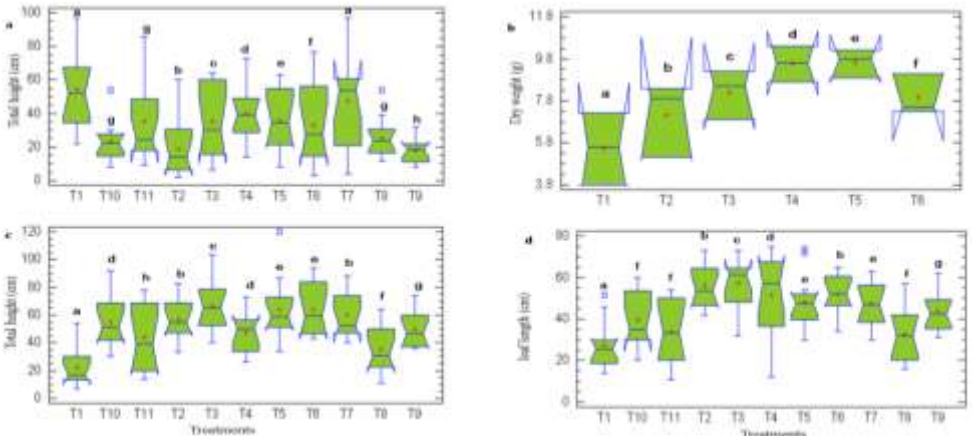

Figure 1:- Differences obtained between biofertilizers treatments evaluated. a-b) total heights and dry weight of *B. brizantha* andc-d) total heights and leaf length of *P.*

máximum (cv. Mombaza). T1: control, T2: fertilized species, T3: CC-azot1, T4: CC-azot2, T5-CC-azot3, T6-CC-azot4, T7-CC-azot5. T8-NFB-azos6, T9-NFB-azos7, T10-NFB-azos8 and T11-NFB-azos9.

In respect with *P. máximum* (cv. Mombaza), it was not detected significative differences ($P \leq 0.05$), however the highest total height obtained was in treatment combined with *Azotobacter*1 (T3) with 68 cm followed by combined with *Azotobacter*4 (T6) with 66 cm (Figure 1c). On the other hand, the treatment T3 obtained a leaf length of 57 cm followed by T2 with 56 cm (Figure 1d).

Experiment evaluation in field

The experiments application of biofertilizers in *P. maximum* grass (cv. Mombaza) don not showed significative statistical differences ($P \geq 0.05$), nevertheless, biological differences were observed due to the inoculation with growing promote bacteria. Moreover, the inoculation of inorganic fertilizer (T2) increased the development of *P. maximum* grass (cv. Mombaza) generating 1.34 kg/m^2 of dry matter production in comparison with application of Azospirillum + Azotobacter (T3) which generated 0.86 kg/m^2. On the other hand, the commercial biofertilizers (T5) increased the development of radicular system in *P. maximum* grass (cv. Mombaza). The weight of radicular system in T5 was 0.19 kg/m^2, furthermore in T2 was 0.15 kg/m^2. For radical volume, it was observed that control showed the best development with 0.15 m^3/L (Table 2).

Table 2:- Results of five treatments with inorganic and biofertilizers applied in *P. maximum* and *B. brizantha* grasses.

Treatments	*P. maximum*						*B. brizantha*				
	DW (kg/m^2)	RW (kg/m^2)	RV (m^3/L)	SD (cm)	TH (m)		DW (kg/m^2)	RW (kg/m^2)	RV (m^3/L)	SD (cm)	TH (m)
1	0.74	0.16	0.12	0.44	2.05		0.70	0.13	0.11	0.45	1.10
2	1.34	0.15	0.13	0.45	2.40		0.74	0.13	0.13	0.46	1.15
3	0.86	0.15	0.15	0.44	2.10		0.89	0.13	0.12	0.50	1.47
4	1.10	0.10	0.14	0.46	2.03		0.90	0.13	0.11	0.47	1.26
5	0.80	0.19	0.17	0.45	2.52		0.99	0.14	0.12	0.49	1.43

1: control, 2: inorganic fertilizer, 3: *Azospirillum+Azotobacter* biofertilizer, 4: Mycorrhizal biofertilizer and 5: commercial biofertilizer. DW: dry weight, RW: radicular weight, RV: radicular volume, SD: stem diameter and TH: total height.

The results obtained in *B. brizantha* showed that inoculation with promoter bacteria showed significative differences ($P \leq 0.05$). The treatment fertilized with *Azotobacte*r + *Azospirillum* (T3) showed 1.47 m of plant heigh. In respect with steam development, the treatment T3 showed the high development with 0.5 cm contrary the control (T1) that showed 0.45 cm of stem development. On the other hand, the commercial biofertilizer (T5) showed statistical differences ($P \leq 0.05$) in dry weight with 0.99 kg/m^2 in respect with inorganic fertilizer (T2) that showed 0.74 kg/m^2. Another variable that showed significative differences between treatments ($P \leq 0.05$) was root weight. The best root weight was observed in the treatment with commercial biofertilizer (T5) with 0.14 kg/m^2.

Discussion:-

The *Azotobacter* had a positive effect in all variables, however, *Azospirillum* do not showed the same effect in our studies. Many authors mentioned that *Azospirillum* provides benefits to the plants if exits high quantity of this bacteria (Zeffa *et al*., 2019; Malinich and Bauer, 2018; Brusamarello-Santos *et al*., 2017). On the other hand, bacteria population increment around the rhizosphere, improve the uptake of minerals by plants (Emami *et al*., 2020; Roriz *et al*., 2020; Fiorentino *et al*., 2018), furthermore increase dry matter and yielding (Hussain *et al*., 2020; Naserzadeh *et al*., 2018; Ghorchiani *et al*., 2018).

The interaction whit those microorganisms around the rhizosphere has been demonstrated by many authors (Santos *et al*., 2018; Mishra *et al*., 2017), for instance, different species have been evaluated with biofertilizers and the responses have been positive (Vasileva *et al*., 2019). In this work was observed that treatment with local strains biofertilizers were low compared with commercial biofertilizer, this could be that these treatments had not mycorrhizal, Aguirre-Medina *et al*. (2009) mentioned that mycorrhizal fungi needs 30 days to grow in radicular

system of plants and therefore increase the process of nutrient and water transportation (Lanfranco *et al*., 2018; Guerrero-Galan *et al*., 2018).

The plants that were inoculated with mycorrhizal fungi had not modification in radicular morphology, however some authors mentioned that plants inoculated with mycorrhizal tends to modify their radicular morphology (Nunes, 2018; da Silva Jr *et al*., 2017).

Conclusions:-

The treatments combined with *Azotobacter*1 (T3) and combined with *Azotobacter*5 (T4) improve the development of *B. brizantha*, the variables that showed better development were leaf length, total heigh and dry weight. On the other hand, the inoculation in Mombaza (*P. máximum*) improve the development in leaf length and total heigh but only in for treatment combined with *Azotobacter*1 (T3).

In respect with mycorrhizal fungi, the treatment with the best response was T5 with better radical volume and dry matter production. The inoculation carried out in field with commercial biofertilizer (T5) improves the production of dry matter and root weight in *B. brizantha*. Accord of our results, treatments with biofertilizers evaluated in *P. máximum* (cv. Mombaza) were equal, however it can be observed better develop in plants, furthermore dry matter production increased up to 22% compared with inorganic fertilizer (T2).

References:-

1. Aguirre-Medina, J. F. 2006. Biofertilizantes microbianos; experiencias agronómicas del programa nacional del INIFAP en México. Libro Técnico Num. 2. Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias. Campo Experimental Rosario Izapa, Tuxtla Chico, Chiapas, México. p.3, 4.
2. Aguirre-Medina, J. F., M. B. Irizar-Garza, A. Duran-Prado, O. A. Grageda-Cabrera, M. A. Peña-del Rio, C. Loredo-Ostil y A. Gutiérrez-Baeza. 2009. Los biofertilizantes microbianos: alternativa para la agricultura en México, Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias. Campo Experimental Rosario Izapa, Tuxtla Chico, Chiapas, México. 80 p.
3. Baweja, P., Kumar, S., & Kumar, G. (2020). Fertilizers and pesticides: Their impact on soil health and environment. In Soil Health (pp. 265-285). Springer, Cham.
4. Brusamarello-Santos, L. C., Gilard, F., Brulé, L., Quilleré, I., Gourion, B., Ratet, P., ... & Hirel, B. (2017). Metabolic profiling of two maize (*Zea mays* L.) inbred lines inoculated with the nitrogen fixing plant-interacting bacteria Herbaspirillum seropedicae and Azospirillum brasilense. PloS one, 12(3), e0174576.
5. Centro Internacional de Agricultura Tropical (CIAT). 1988. Simbiosis leguminosa-rizobio; manual de métodos de evaluación, selección y manejo agronómico. Ed. Rev. Proyecto CIAT-UNDP de evaluación, selección y manejo de la simbiosis leguminosa-rizobio para aumentar la fijación de nitrógeno. Sección de microbiología de suelos del programa de pastos tropicales y sección microbiología de suelos del programa de frijol. Cali, Colombia. p. 178.
6. Chávez-Herrera, E., Hernández-Esquivel, A. A., Castro-Mercado, E., & García-Pineda, E. (2018). Effect of *Azospirillum brasilense* Sp245 lipopolysaccharides on wheat plant development. Journal of Plant Growth Regulation, 37(3), 859-866.
7. Coniglio, A., Mora, V., Puente, M., & Cassán, F. (2019). *Azospirillum* as biofertilizer for sustainable agriculture: *Azospirillum brasilense* AZ39 as a model of PGPR and Field traceability. In Microbial Probiotics for Agricultural Systems (pp. 45-70). Springer, Cham.
8. da Silva Jr, J. M. T., Mendes Filho, P. F., Gomes, V. F. F., de Almeida, A. M. M., & Garcia, K. G. V. (2017). Morphological pattern of colonization by mycorrhizal fungi and the microbial activity observed in Barbados cherry crops/Padrao morfologico de colonizacao por fungos micorrizicos arbusculares e atividade microbiana em cultivos de acerola. Ciencia Rural, 47(12).
9. Emami, S., Alikhani, H. A., Pourbabaee, A. A., Etesami, H., Motasharezadeh, B., & Sarmadian, F. (2020). Consortium of endophyte and rhizosphere phosphate solubilizing bacteria improves phosphorous use efficiency in wheat cultivars in phosphorus deficient soils. Rhizosphere, 14, 100196.
10. Etminani, F., & Harighi, B. (2018). Isolation and identification of endophytic bacteria with plant growth promoting activity and biocontrol potential from wild pistachio trees. The plant pathology journal, 34(3), 208.
11. Fiorentino, N., Ventorino, V., Woo, S. L., Pepe, O., De Rosa, A., Gioia, L., ... & Rouphael, Y. (2018). Trichoderma-based biostimulants modulate rhizosphere microbial populations and improve N uptake efficiency, yield, and nutritional quality of leafy vegetables. Frontiers in plant science, 9, 743.

12. García, J. O., V. M. Moreno, C. L. Rodríguez, A. H. Mendoza y N. P Mayek. 2006. Biofertilización con *Azospirillum brasilense* en sorgo en el norte de México. Centro de Biotecnología Genómica, Instituto Politécnico Nacional. Reynosa Tamaulipas, México. Vol. 32.
13. García-Sánchez, M., Silva-Castro, G. A., Sanchez, A., Arriagada, C., & García-Romera, I. (2021). Effect of arbuscular mycorrhizal fungi and mycoremediated dry olive residue in lead uptake in wheat plants. Applied Soil Ecology, 159, 103838.
14. Ghorchiani, M., Etesami, H., & Alikhani, H. A. (2018). Improvement of growth and yield of maize under water stress by co-inoculating an arbuscular mycorrhizal fungus and a plant growth promoting rhizobacterium together with phosphate fertilizers. Agriculture, Ecosystems & Environment, 258, 59-70.
15. Guerrero-Galan, C., Houdinet, G., Calvo-Polanco, M., Bonaldi, K. E., Garcia, K., & Zimmermann, S. D. (2018). The role of plant transporters in mycorrhizal symbioses. Advances in Botanical Research, 87, 303-342.
16. Hashem, A., Abd_Allah, E. F., Alqarawi, A. A., & Egamberdieva, D. (2018). Arbuscular mycorrhizal fungi and plant stress tolerance. Plant microbiome: stress response, 81-103.
17. Hussain, A., Zahir, Z. A., Ditta, A., Tahir, M. U., Ahmad, M., Mumtaz, M. Z., ... & Hussain, S. (2020). Production and implication of bio-activated organic fertilizer enriched with zinc-solubilizing bacteria to boost up maize (*Zea mays* L.) production and biofortification under two cropping seasons. Agronomy, 10(1), 39.
18. Jose, S., & Dollinger, J. (2019). Silvopasture: a sustainable livestock production system. Agroforestry systems, 93(1), 1-9.
19. Kashyap, A. S., Pandey, V. K., Manzar, N., Kannojia, P., Singh, U. B., & Sharma, P. K. (2017). Role of plant growth-promoting rhizobacteria for improving crop productivity in sustainable agriculture. In Plant-microbe interactions in agro-ecological perspectives (pp. 673-693). Springer, Singapore.
20. Keesing, F., Ostfeld, R. S., Okanga, S., Huckett, S., Bayles, B. R., Chaplin-Kramer, R., ... & Allan, B. F. (2018). Consequences of integrating livestock and wildlife in an African savanna. Nature Sustainability, 1(10), 566-573.
21. Kılıçalp, N., Avcı, M., Hızlı, H., Hatipoğlu, R., & Ayaşan, T. (2018). Botanical composition and in situ dry matter degradability of legume-grass mixture pasture fertilized with different amounts of nitrogen. Turkish Journal of Agriculture-Food Science and Technology, 6(1), 16-21.
22. Koli, P., Bhardwaj, N. R., & Mahawer, S. K. (2019). Agrochemicals: harmful and beneficial effects of climate changing scenarios. In Climate Change and Agricultural Ecosystems (pp. 65-94). Woodhead Publishing.
23. Kour, D., Rana, K. L., Yadav, A. N., Yadav, N., Kumar, M., Kumar, V., ... & Saxena, A. K. (2020). Microbial biofertilizers: Bioresources and eco-friendly technologies for agricultural and environmental sustainability. Biocatalysis and Agricultural Biotechnology, 23, 101487.
24. Lanfranco, L., Fiorilli, V., & Gutjahr, C. (2018). Partner communication and role of nutrients in the arbuscular mycorrhizal symbiosis. New Phytologist, 220(4), 1031-1046.
25. Legge, S., Smith, J. G., James, A., Tuft, K. D., Webb, T., & Woinarski, J. C. (2019). Interactions among threats affect conservation management outcomes: Livestock grazing removes the benefits of fire management for small mammals in Australian tropical savannas. Conservation Science and Practice, 1(7), e52.
26. Mącik, M., Gryta, A., & Frąc, M. (2020). Biofertilizers in agriculture: An overview on concepts, strategies and effects on soil microorganisms. Advances in Agronomy, 162, 31-87.
27. Malinich, E. A., & Bauer, C. E. (2018). The plant growth promoting bacterium Azospirillum brasilense is vertically transmitted in *Phaseolus vulgaris* (common bean). Symbiosis, 76(2), 97-108.
28. Mishra, J., Singh, R., & Arora, N. K. (2017). Alleviation of heavy metal stress in plants and remediation of soil by rhizosphere microorganisms. Frontiers in microbiology, 8, 1706.
29. Modesto, R., Pinheiro, C. S. C., Pereira, L. E. T., Carraschi, I. V. T., Rocha, C. O., & Herling, V. R. (2018). Population density and average tiller weight of marandu grass pastures in response to the use of biofertilizers. In 55a Reunião Anual da Sociedade Brasileira de Zootecnia, 28° Congresso Brasileiro de Zootecnia, Goiânia, Brasil, 27 a 30 de agosto de 2018. Sociedade Brasileira de Zootecnia-SBZ, Associação Brasileira dos Zootecnistas.
30. Muciño-Álvarez, M., Albarrán-Portillo, B., López-González, F., & Arriaga-Jordán, C. M. (2021). Multi-species pastures for grazing dairy cows in small-scale Dairy systems in the highlands of Mexico. Tropical Animal Health and Production, 53(1), 1-9.
31. Naserzadeh, Y., Kartoolinejad, D., Mahmoudi, N., Zargar, M., Pakina, E., Heydari, M., ... & Kavhiza, N. J. (2018). Nine strains of Pseudomonas fluorescens and P. putida: Effects on growth indices, seed and yield production of *Carthamus tinctorius* L. Research on Crops, 19(4), 622-632.

32. Nunes, J. L. S. (2018). Anatomic and Morphologic Relations Developed Between Plants Roots and Arbuscular Mycorrhizal Fungi (AFM) During The Establishment of Mycorrhizal Symbiosis: A Review. Open Acc J Agri Res: OAJAR-100010, 2018(02).
33. Pajčin, I. S., Vlajkov, V., Dodić, J., Jokić, A., & Grahovac, J. (2021). Biotechnological production of plant inoculants based on nitrogen-fixing bacteria. Journal on Processing and Energy in Agriculture, 25(2), 56-63.
34. Raklami, A., Tahiri, A. I., Bechtaoui, N., Pajuelo, E., Baslam, M., Meddich, A., & Oufdou, K. (2021). Restoring the plant productivity of heavy metal-contaminated soil using phosphate sludge, marble waste, and beneficial microorganisms. Journal of Environmental Sciences, 99, 210-221.
35. Roriz, M., Carvalho, S. M., Castro, P. M., & Vasconcelos, M. W. (2020). Legume biofortification and the role of plant growth-promoting bacteria in a sustainable agricultural era. Agronomy, 10(3), 435.
36. Santos, M. L. D., Berlitz, D. L., Wiest, S. L. F., Schünemann, R., Knaak, N., & Fiuza, L. M. (2018). Benefits associated with the interaction of endophytic bacteria and plants. Brazilian archives of biology and technology, 61.
37. Thakur, N., Sharma, M., & Sharma, M. (2021). Phytoremediation-a green technology adapted to eradication of harmful heavy toxic metals from contaminated soil. Journal of Innovative Agriculture, 8(1), 26-31.
38. Vasileva, E. N., Akhtemova, G. A., Zhukov, V. A., & Tikhonovich, I. A. (2019). Endophytic microorganisms in fundamental research and agriculture. Ecological genetics, 17(1), 19-32.
39. Williams, D. R., Alvarado, F., Green, R. E., Manica, A., Phalan, B., & Balmford, A. (2017). Land-use strategies to balance livestock production, biodiversity conservation and carbon storage in Yucatán, Mexico. Global Change Biology, 23(12), 5260-5272.
40. Zeffa, D. M., Perini, L. J., Silva, M. B., de Sousa, N. V., Scapim, C. A., Oliveira, A. L. M. D., ... & Azeredo Goncalves, L. S. (2019). *Azospirillum brasilense* promotes increases in growth and nitrogen use efficiency of maize genotypes. PLoS One, 14(4), e0215332.
41. Zhang, H., Xu, N., Li, X., Long, J., Sui, X., Wu, Y., ... & Sun, G. Y. (2018). Arbuscular mycorrhizal fungi (*Glomus mosseae*) improves growth, photosynthesis and protects photosystem II in leaves of Lolium perenne L. in cadmium contaminated soil. Frontiers in plant science, 9, 1156.