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REVIEW ARTICLE

Rhizobium Biofertilizer (Okadin) Production and Future Prospects in Sudan

Randa H. Elsalahi*, Somaya S. Mohamed, Ashraf M. Sherif and Awad G. Osman

Environment, Natural Resources and Desertification Research Institute, National Centre for Research, P.O. Box 6096, Khartoum, Sudan

*Corresponding Author: randa_9123@hotmail.com

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Abstract

This article summarizes all aspects concerning production of the *Rhizobium* biofertilizer "Okadin" taking place at the Environment, Natural Resources and Desertification Research Institute (ENDRI), National Centre for Research (NCR), Khartoum, as a pivotal activity evaluated from the viewpoint of its actual status regarding production, expansion and the future roadmap. Research carried out to evaluate the effects of *Rhizobium* inoculation on different leguminous crops in Sudan were reviewed; besides brief historical accounts on Okadin (inoculant) production including, selection of the efficient bacterial isolates, best carrier from the abundant local materials, storage conditions, commercial production, marketing and the future prospects. The efforts done to disseminate the technology of Okadin application among farmers and producers in public and private sectors through various routes, particularly training courses, extension programs and on-farm trials, were highlighted. Moreover, the economic benefits due to *Rhizobium* inoculation were equally stressed. Such economic values were represented by the increasing demand from 142 to 6008 ha/year between 1992 and 2015, which has been reflected in expanding Okadin production and commercialization. Consequently, an improvement in yield quantity (ranged between 9.5 - 50%) as well as yield quality of several leguminous crops, were attained. It is concluded that local habitats are rich in a diversity of beneficial microorganisms with vital potentialities in sustainable agriculture, which entails extensive research works to be screened and utilized efficiently in producing economically feasible and environmentally sound biofertilizers.

Keywords: Fertilizer, efficiency, carrier, legumes, economic, quality.

Introduction

Sudan's economy depends mainly on agriculture, though the cultivated area is far below that of total arable land due to financial and technical limitations. Cultivation of legumes is practiced all over the country, traditionally, and largely restricted to smallholder farmers. Mechanization is used mainly for land preparation, while synthetic pesticides and fertilizers are employed. Large-scale production of legumes was practiced only three decades ago with cluster bean (*Cyamopsis tetragonoloba*) in central Sudan for guar gum production, and earlier with soybean (*Glycine max*) for oil extraction and export (Abdelgani *et al*., 1998). Recently, vast areas are cultivated with alfalfa due to the expansion of investments in fodder cultivation for export. Many leguminous plant species form a symbiotic association with types of bacteria that are collectively termed “rhizobia”. The rhizobia fix inert atmospheric nitrogen (N_2) into biologically useful forms within legume root nodules in a process called “Biological N_2 fixation” (BNF). This symbiotic association is the largest natural source of the N cycled to sustain natural systems. In addition, BNF by grain and forage legumes plays an important role in enriching the pools of soil N for non-N_2-fixing subsequently grown crops as part of a strategic cropping sequence or rotation (Iannetta *et al*., 2016).

Agricultural research in Sudan started very early last century, and the importance of introducing leguminous crops to the rotation in Gezira Agricultural Scheme was realized and practiced since the early thirties (FAO, 2006). Sporadic research activities on BNF in Sudan started in the sixties last century. In 1983, a pilot demonstration project was launched for increasing legume protein production through BNF that was funded by the United Nations Environment Program (UNEP). The participating bodies in the project were the National Centre for Research (NCR), Agricultural Research Corporation (ARC), and the University of Khartoum. It was a successful project that led to the establishment of a central laboratory for inoculant production, which has been developed into the Biofertilization Department (BFD), Environment and Natural Resources Research Institute (ENRRI) at NCR. Currently, it is the Biofertilization Unit (BFU), Department of Biopesticides and Biofertilizers, Environment, Natural Resources and Desertification Research Institute (ENDRI), NCR. Now it is the only institution in the country producing legume *Rhizobium* inoculant, which is registered at the intellectual property authority. The product is known as “Okadin” after the Arabic word “Okad” which means “nodules”. The commercial production of Okadin inoculant has started since 1992 (Elhassan *et al*., 2010).

A huge number of isolates are authenticated and tested for their efficiency in N fixation and yield improvement of leguminous crops (food and forage). Such strains and isolates are formulated into carrier based *Rhizobium* inoculants after thorough experimentation on the potential of different local materials to serve as suitable carriers. Expansion in demand was promoted by mutual efforts of the BFU and the extension specialists through training, demonstration farms and dissemination. Consequently, annual production of rhizobial inoculants witnessed a leap frog increase from several kilograms to several tones. Such success inspired the endeavor of new approaches in biofertilization and environmental biotechnology as general. This paper reviewed all aspects concerning production and research development of the *Rhizobium* biofertilizer (Okadin) in Sudan.

Research on rhizobial inoculants production

Researchers in BFU have worked hard to promote the use of rhizobial inoculants as an alternative to the highly expensive and environment deteriorating chemical N-fertilizers. Extensive research concerning inoculant production, application and improvement are being conducted for a long period of time.

Rhizobial strain selection: Selection of suitable rhizobial strain for each leguminous crop is the first important step for inoculant production. Therefore, for each leguminous crop several strains and isolates of rhizobia were firstly screened for their capability in nodulation and N_2-fixation in the glasshouse. After that, each selected strain was tested as an inoculant for its capability of nodulation, N_2-fixation and yield response in several fields. Positive responses (increase in production, improvement of quality and soil N content) have been recognized and documented in most experiments carried out by the researchers of the BFU for the major leguminous crops in some parts of the country (Fig. 1 & 2). Also, the conducted research came out with a number of efficient

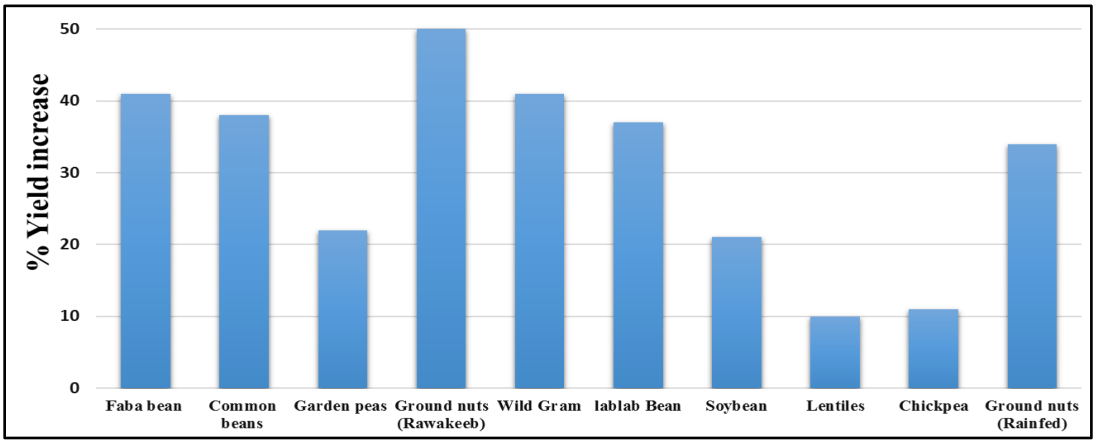

Fig. 1. Yield increase in some leguminous crops due to *Rhizobium* inoculation.
* Source: Elhassan *et al*. (2010).

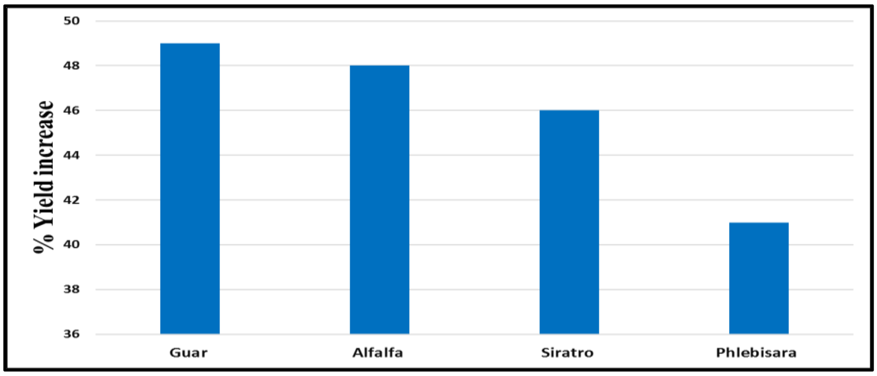

Fig. 2. Yield increase in some leguminous forage crops due to *Rhizobium* inoculation.
* Source: Elhassan *et al*. (2010).

strains that were identified, preserved and deposited in the BFU for inoculant production. Studies revealed that several *Rhizobium* stains have the capacity of improving productivity of leguminous crops and soil fertility in dry lands (Abdelgani *et al*., 2003), saline soils (Elsheikh, 1998a, and Elsheikh, 2001) and could tolerate up to 50°C moist heat for 15 minutes (Habish and Kheiri, 1968, and Haddad, 1984). *Rhizobium* strains are continuously provided on request to students in universities or researchers from different research institutions for experimentation purposes.

Carrier selection: Selection of the inoculant carrier is the second concern for rhizobial inoculant production. A microbial carrier has to comply with several requirements, viz. non-toxic to rhizobia or plants, of around neutral pH, has a good moisture holding capacity, available in fine particle size for better adherence to seeds, supports microbial growth and proliferation during storage, of the least contamination liability and at ample quantities at moderate cost (Somasegaran and Hoben, 1994). Peat-based and oil-based inocula have been tested for *Rhizobium* survival on groundnut seeds by Haddad (1984), who stated that both inocula gave counts exceeding 10^7 rhizobia $g^{-1}$with peat being superior. Peat is generally used as a microbial carrier in many countries and since it is not available in Sudan, researchers were prompted to evaluate several local materials to be used as microbial carriers; sterilization and shelf life were also considered.

As pioneer researchers tackling the laborious task of finding suitable rhizobial carrier from local materials in Sudan, El Shafie and El Hussein (1991) tested the suitability of charcoal and filter mud as carriers for the survival of *Rhizobium* isolates at low and high temperatures. Both carriers were found to be suitable and maintained high rhizobia populations (1.0×10^8 rhizobia g^{-1}). Abdelrahim *et al*. (2005) assessed the suitability of locally available materials such as Nile silt, bagasse, groundnut shells and charcoal powder as carriers for rhizobia (Fig. 3). Nile silt alone was previously tested by Elgaali *et al*. (2002). Also, Osman and Mohamed (2002) studied the efficiency of using sterile and non-sterile charcoal powder and Nile silt as carriers for rhizobial inoculants. All tested substances proved efficiency, but charcoal was found to be superior in terms of rhizobial counts, availability and abundance besides its high water holding capacity, and its least contamination liability and storage ability of 60 days at room temperature as shown in Fig. 4 and 5.

Sterilization usually eliminates contaminants that might out-compete rhizobia or even secrete toxic substances that lead to death of *Rhizobium* or impair their metabolic activities. In addition, sterilization maintains and improves viability of the impregnated rhizobia (Swelim *et al*., 2010). Therefore, several sterilization methods were conducted to choose the suitable method for charcoal sterilization. Autoclaving at 121˚C and 15 lb/in^2 for one hour proved to be effective in sustaining the recommended rhizobial counts in charcoal (Elsalahi, 2003) for up to two months. Figure 4 & 5 show rhizobial counts in autoclave sterilized and non-sterilized charcoal carrier (Osman and Mohamed, 2002). However, oven sterilization of charcoal at 100˚C for two hours is now adopted for commercial production of Okadin biofertilizer as it proved to be efficient and much convenient (Fig. 4 and 5).

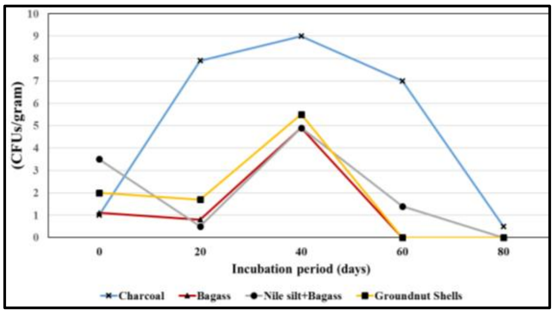

Fig. 3. Shelf life of different *Rhizobium* carriers.

*Source: Abdelrahim *et al.*, 2005.

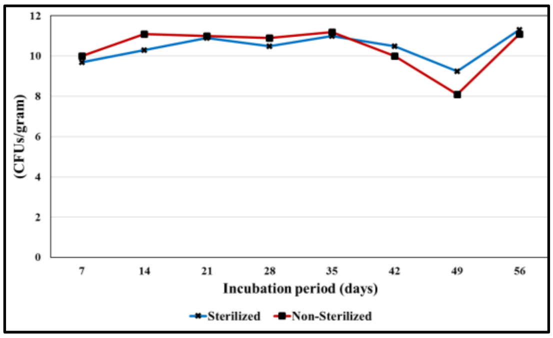

Fig. 4. Rhizobial counts (ENRR 11) in oven sterilized and non-sterilized charcoal carrier.

*Source: Osman and Mohamed, 2002.

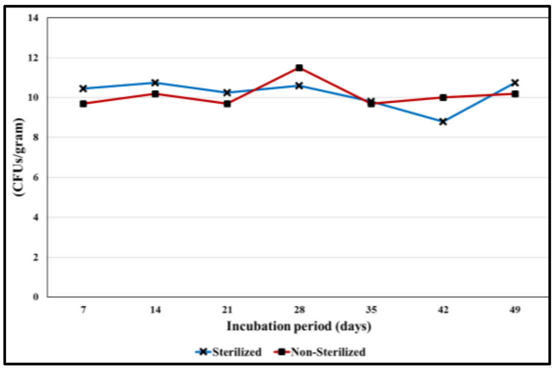

Fig. 5. Rhizobial counts (TAL 380) in oven sterilized and non-sterilized charcoal carrier.

*Source: Osman and Mohamed, 2002.

Inoculant formulation and quality control
Charcoal fragments are cleaned from dirt and debris, milled and sieved to pass 1 mm mesh screen, then the pH of the charcoal powder is adjusted to 7 and mixed thoroughly with the recommended volume of inoculum (>10^9CFU/ml) to maintain 40% moisture content. One kilogram inoculant is then packed in polyethylene bags and sealed. The required information such as type and weight of content, target legume, directions of use, storage conditions, shelf life, and the logo and location of the manufacturing institution are printed on the polyethylene bags. As a routine quality control measure conducted by the BFU, the impregnated carriers are tested for their viable bacterial counts at the beginning and at various stages during storage, transport and application. Besides nodulation and nitrogen fixation potential for the evaluation of different rhizobial strains, molecular techniques are now on the way to be employed in the BFU as efficient and advanced tools for quality control where strains are being checked for the persistence of the N_2 fixing traits. In Sudan, inoculants can safely be stored for 6–10 weeks under ambient temperature (up to 37°C) (El Shafie and El Hussein, 1991).

Efficiency of *Rhizobium* inoculation
According to the research carried in the BFU and in several universities and research institutions, *Rhizobium* biofertilizer have pronouncedly improved both yield and quality of most cultivated food leguminous crops (Fig. 1), viz. ground nut (*Arachis hypogaea*) (Abdelgani and Ibrahim, 2001, and Mohammed and Abdalla, 2013), chickpea (*Cicer arietinum*) (Abdalla *et al*., 2011; Abdalla *et al*., 2013, and Hassan and Mohamed, 2015), pigeon pea (*Cajanus cajan*) (Mahdi and Mustafa, 2005; Elsoni and Osman, 2011, and Osman *et al*., 2011), soybean (*Glycine max*) (ELsheikh *et al*., 2009), common bean (*Phaseolus vulgaris*) (Osman *et al.,* 1996a, and Mohamed Ahmed *et al*., 2009), lentil (*Lens culinaris*) (Elamin and Abdelgani, 2001), garden pea (*Pisum-sativum*) (Mohamed *et al*., 1996) and fenugreek (*Trigonella foenum graecum*) (Abdelgani *et al.,* 2003). In the same instance, Mahdi and Mustafa (2005) in an investigation indicated that although *Vigna unguiculata, Crotalaria saltiana* and *Cassia occidentalis* were naturally nodulated in Sudan, inoculation by introduced or locally-isolated hizobia had improved nodulation and dry matter production in these crops. However, Ali (2003) reported that *Rhizobium* inoculation had no significant effects on groundnut production, neither on the number of pods nor on yield of common beans (Elballa *et al*., 2004).

Regarding faba bean (*Vicia faba*) response to inoculation, a great number of studies had been carried out where results showed that *Rhizobium* inoculation had remarkably improved nodulation, yield, yield attributes and seed quality (Osman and Mohamed, 1996; Osman *et al*., 1996b; Elsheikh, 1998b; Elsheikh and Ahmed, 2000; Osman *et al*., 2010; Osman and Abd-Elaziz, 2010; Mohamed and Babiker, 2012, and Rugheim and Abdelgani, 2012). Inoculation also decreased faba bean infection by Bean Yellow Mosaic Virus (Osman, and Elsheikh, 1994, and Babiker *et al*., 1995) and Broad Bean Mottle Bromovirus (Elsheikh and Osman, 1995) as well. Moreover, *Rhizobium* inoculation and nitrogen fixation helped in alleviation of salinity effects on faba bean (Elsheikh and Osman, 2002).

Generally, fodder legumes have been far less studied compared to food legumes (Fig. 2). However, alfalfa (*Medicago sativa*) has been extensively studied and showed remarkable increase in fodder production and improved quality in response to *Rhizobium* inoculation (Mohamed and Osman, 1996); crude protein and phosphorus contents were increased by 26.9% and 32.3%, respectively, during the winter season (Abusuwar and Ahmed, 2003). Moreover, clitoria (*Clitoria ternatea*), phillipesara (*Phaseolus trilobus*), siratro (*Macroptilium atropurpureum*) (Abdelgani *et al*., 1997) and lablab bean (*Lablab purpureus*) (Hassan and Abdelgani, 2009) are among the fodder legumes mostly studied. Cluster bean (*Cyamopsis* *tetragonoloba*), though introduced to Sudan in 1939, gained attention only recently and large scale production of guar gum only started early last decade. Being a promising crop, it is attracting numerous research works as with respect to its response to inoculation. *Rhizobium* inoculation of guar had significantly increased shoot and root dry weights as well as grain yield (Abdelgani *et al*., 1998, and Mahdi and Mustafa, 2005).

The BFU had conducted a survey on nodulation of wild legumes growing in different parts of the country. The objective was to explore the probable efficiency of indigenous wild legume *rhizobia* in nodulating, N_2 fixing and improving production of compatible cultivated food and forage legumes. So far, *Rhizobium* strains are isolated from *Rhyncosia minima* and *Alysicarpus rugosus*. The isolates had been screened and classified according to their compatibility with some crop legumes (Abdelgani *et al*., 1997).

Regarding response of tree legumes to rhizobia, a study of Rhizobial cross inoculation groups of *Faidherbia albida*, *Acacia nilotica*, *Acacia Senegal*, *A. tortilis*, *A. seyal* and *A. melifera*, revealed that the frequency of nodulation and total nitrogen content were maximized when each individual plant species was inoculated with its own isolate of *Rhizobium*. Moreover, inoculation of *Acacia* spp. has been improved when combining NPK fertilizer with *Rhizobium* (Elhassan *et al*., 2010).

***Rhizobium* and co-inoculation**

In terms of increasing the efficiency and improving performance of rhizobial inoculants, several studies concerning co-inoculation have been carried out where non-symbiotic nitrogen fixing and phosphorus and potassium solubilizing microbes had been introduced with rhizobia. Even several strains of *Rhizobia* could be applied as co-inoculants. The technique has revealed promising results in several experiments. *Bradyrhizobium* and/or mycorrhizal inoculation significantly increased the ash, crude fiber, in vitro protein digestibility (IVPD) and tannin content of groundnut (Elsheikh and Mohamedzein, 1998). Yield and uptake of N_2 and P by groundnut were significantly higher in the treatments receiving both *Rhizobium* and phosphobacterium inoculants than individual application of either inoculant (Mohamed and Abdalla, 2013). In another study, Ahmed *et al*. (2005) reported that co-inoculation of groundnuts with *Bradyrhizobium* and *Azospirillum* induced an increase in yield corresponding to 90% increase caused by 86 kg N/ha. *Rhizobium* and *Bacillus megaterium* var. *phosphaticum* co-inoculation significantly ($p<0.05$) increased yield, 100 seed weight, ash, fat, protein content and cookability of chickpea seeds in a field experiment conducted in Gezira State, central Sudan (Abdalla *et al*., 2013). In the same context *Rhizobium* co-inoculated with *Azospirillum brazilense* significantly increased shoot N content of both faba bean and chickpea (Table 1).

Table 1. Effect of *Rhizobium* and *Azospirillum* inoculation on shoot nitrogen content (%) of chickpea and faba bean.

Treatment	**Chickpea**	**Faba bean**
Control	1.26	1.12
Rhizobium	1.54	1.40
Azospirillum	1.40	1.75
Rhizobium + Azospirillum	1.75	2.07
L.S.D (5%)	0.15	0.15

* Source: Mohamed, 2006

Considering *Rhizobium* multi-stain inoculation as a candidate of co-inoculation, then we can highlight the results of Mohamed Ahmed *et al*. (2009), who found that *Rhizobium* multi strain inoculation significantly increased dry weight of shoots, roots and nodules and number of nodules of chickpea. Mahdi and Mustafa (2005) working on forage legumes found that *Bradyrhizobium* inoculation significantly improved nodulation and dry matter production of guar particularly by locally isolated bradyrhizobia. *Rhizobium melilotti*, Vesicular Arbuscular-mycorrhizae, and their combinations significantly increased forage fresh yields by 42%, 60% and 65%, respectively (Abusuwar and Ahmed, 2003).

Economic benefit

Several studies tackling economic feasibility of using rhizobial fertilizers have been carried out in the BFU. Results of the financial analysis of two indigenous faba bean cultivars, "Silaim" and "Agabat" showed that *Rhizobium* inoculation and nitrogen fertilization were financially feasible, but the net returns obtained from *Rhizobium* inoculation were by far greater than those of urea fertilization for the two cultivars (Osman *et al.*, 1996b). Table 2 shows the potential saving that could be obtained by cultivation of the areas devoted to groundnut using rhizobial inoculation instead of urea as estimated from the yield obtained in response to inoculation.

In an experiment conducted by Mohamed and Osman (1996), *Rhizobium* inoculation was found to promote yield of alfalfa and produced the same dry fodder yield compared to urea application. However, cost wise it was found to be remarkably cheaper making a 770 SDG (40 USD approximately) per hectare saving in cost of production, as estimated by current prices (Table 3).

Promotion of rhizobial inoculants

Extension and on-farm trials: The BFU used to conduct intensive training and extension programs with the objective of introducing BNF technology to agricultural extension workers and other personnel involved in agricultural farming. Besides, the strong link with the Extension Departments of the federal and State Ministries of Agriculture made it possible for the BFU to conduct on-farm trials to serve as demonstration farms. This approach had remarkably promoted Okadin production and led to an increase from 338 kg in 1992 to 14300 kg in the year 2015 to meet the increasing demand. One-day seminars and lectures used to be frequently held at villages close to the farmers' fields by staff members of the BFU and previously trained agricultural extension workers.

Table 2. Comparison of groundnut production cost using Okadin vs. Urea fertilization.

Parameter	Groundnuts	
	2010	**2015***
Total cultivated area (feddan) rainfed sector	3,383,000	6,417,000*
Average productivity kg/feddan	211.6	360*
Total production million tons (without fertilization)	715	2,310
Total production (tone) (using urea) million tons	830 16%	2,679 16%
Total production (tone) (using Okadin) million tons	959,000 34%	3,095 34%
Cost of urea fertilization (SDG)	287,555,000	2,566,800,000
Cost of Okadin fertilization (SDG)	50,745,000	513,360,000
Difference in total cost (SDG)	236,810,000	2,053,440,000

* CBOS Central Bank of Sudan (2015)

Table 3. Alfalfa productivity and cost using Okadin vs. Urea fertilization.

Treatments	Green fodder tone/ha	Dry fodder tone/ha	Cost SDG
Control	0.76	0.22	-
Urea kg/ha	1.11	0.28	960
Okadin	0.98	0.28	190

* Source: ENRRI Report, 1996

Training and dissemination: The BFD used to conduct training courses in rhizobial inoculation technology for undergraduate and postgraduate students. The BFU also prepares pamphlets and brochures on the advantages and application of legume inoculants. Due to shortage in mass media outreach extension programs and transport facilities, the spread of knowledge about BNF technology is a slow process among farmers in remote areas and only personal contacts are serving this purpose in the meantime. Farmer-to-farmer flow of information also helps to disseminate information on use of inoculants.

Expansion in *Rhizobial* inoculant production

Sudan witnesses an agricultural revolution aiming at increasing production, horizontally and vertically. According to the National Comprehensive Strategic Plan of the country, the area devoted to legumes cultivation is annually increasing. This necessitates a corresponding increase of fertilizers use that eventually increases the cost of production and soil deterioration. Hence, adoption of inoculation programs for increasing legume production can be achieved through the low cost environmentally-friendly BNF technology, and the importance of inoculation appears obviously in situations where leguminous crops are introduced into a new area. Although, annual production of Okadin is steadily increasing, but it isn't satisfactory to meet the actual demand (Fig. 6).

Biofertilizers' future prospects

Liquid inocula: The recent expansion in large mechanized fodder production schemes brought about a very large demand on liquid rhizobial inocula that are much convenient in application and highly cost effective incurring least labor cost. Intensive research is being carried out in the BFU tackling different polymer additives in order to promote *Rhizobium* viability and expand shelf life of inocula.

NPK biofertilizer: The pronounced success of research on *Rhizobium* isolation, carrier formulations and Okadin production, owing to the increasing positive perception and attitude towards adoption of the technology, researchers were encouraged to extend research into areas of non-symbiotic nitrogen fixing bacteria and phosphate and potassium solubilizing bacteria as well. Research in this area came out with fairly consistent and promising results that inspired attention to go for an entirely microbial NPK fertilizer.

Piloting: Since facilities at the BFU are mainly planned for research and training activities, the unit is investigating the possibility that local investors would take over the process of production, distribution and marketing of Okadin which will save time and facilities for the unit to concentrate on research, training and quality control aspects of microbial inocula. As a step forward, a pilot unit for Okadin production has been established recently at Soba area, south Khartoum, as a development project financed by the Ministry of Finance and National Economy.

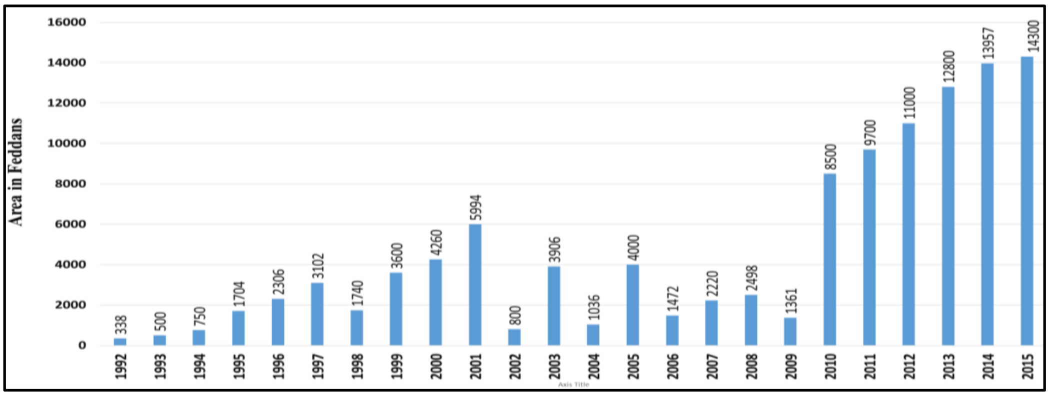

Fig. 6. Annual areas cultivated using *Rhizobium*-based biofertilizer during 1992-2015.

* Source: Elsalahi *et al*. (2015).

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