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RESEARCH ARTICLE

POTENTIAL OF *JATROPHA CURCAS* SEEDCAKE AS SOURCE OF RENEWABLE INSECTICIDE AND BIOFERTILIZER FOR TWO FOOD CROPS LEGUMES: A CASE STUDY OF PEANUT AND BAMBARA GROUNDNUT

Kabé Hinlibé Karka^{1,2, *}, Gomoung Doloum^{4,2}, Augustin Schinzoumka Passannet⁵, Tchuenteu Lucien², Tchobsala³, Megueni Claudilde² and Njintang Yanou Nicolas²

¹Higher National Institute of Agronomic Sciences and Food Technologies of Laï,
Department of Agronomic Sciences, Tchad

²University of Ngaoundéré, Faculty of Science, Department of Biological Sciences, BP: 454, Cameroon

³University of Maroua, Faculty of Sciences, Department of Biological Sciences, B P: 814, Cameroon

⁴University of Sarh, Faculty of Sciences, Department of Biological Sciences, B P: 105, Tchad

University of Pala, Technical Faculty of Sciences and Technology, Department of the Life Sciences and of Ground, LP 28 Pala, Chad

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*Corresponding author:

Kabé Hinlibé Karka

ABSTRACT

The study was carried out in the field, in the southwest of Chad during the 2018 agricultural campaign. The objective of this work was to evaluate the biocidal and fertilizing effects of residues resulting from the extraction of *Jatropha curcas* oil on the control of pests of legume pods in the field. The experimental device was a split plot (2x4) with two factors: the first factor is the legume species (*Vigna subterranea* and *Arachis hypogaea*) and the second the treatments (*J. curcas* seedcake, *J. curcas* seedpowder, urea or positive control and control or negative control). The tests were repeated four times. Data on growth parameters, legume production and soil characteristics were collected. The results obtained indicated that the treatments had a significant effect ($P < 0.05$) on the growth, development, dry biomass and seed yield of the plants. They have made it possible to raise the level of soil fertility to make it more profitable for agricultural activities. The insecticidal and insect repellent potential contained in the seeds of *J. curcas* helped to limit the damage caused by centipedes on peanut and bambara groundnut pods. This agricultural practice helps to widen the way for the practice and promotion of organic farming.

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INTRODUCTION

Jatropha curcas, a shrub of the Euphorbiaceae, is an oilseed plant that seeds can contain up to 40% of the total dry mass. In order to cope with the growing warming caused by the massive release of CO₂ into the air due to the combustion, mainly of fossil oil by industries, efforts are being made to mitigate it (Tchobsala et al., 2016). The search for substitutes for these fossil fuels has led to renewed interest in oilseeds. In order not to create competition with food production intended for animal consumption, in particular human consumption, non-food oil plants are particularly targeted (Kabe et al., 2020).

Thus, *Ricinus communis* and *Jatropha curcas* are retained. They are easy to grow because of their diverse adaptation to soils and climates (Derogoh et al., 2018). In addition to the biofuel use of the oil, it contains very toxic essences that make it a biopesticide for effective biological control against food crop pests in fields and stocks (Kasuya, 2013). The efficacy of the toxicity of *J. curcas* exploited for insecticide and nematocidal purposes has very often given positive results on food crop pests (Orwa et al., 2009; Abdoulaye, 2018). The production of the natural biopesticide from the plant is within the reach of the average farmer to protect crops in the field.

Conventional pesticides are often not affordable to farmers and have a negative effect on the natural ecosystem (Nwaga, 2009; Abdoul *et al.*, 2013). Oil extraction makes it possible to obtain cake with a high protein content (60% of dry matter). It remains very toxic because of the rest of the toxic substances in the seed, in particular the phorbol ester, which are not heat-mobile. Detoxification of this meal makes it an excellent protein supplement for farm animals (King, 2009; Tchobsala, 2013). Hanging of this poisonous cake can protect pods and some bulbs from soil insects, thereby increasing crop yields. This contribution constitutes the use of organic fertilizers to avoid disturbing the natural ecosystem, often caused by chemical fertilizers. These are organic farming practices with no impact on the environment (Jamaludin and Singh, 2006; Jacquet, 2009). The cake obtained after oil extraction is an excellent organic fertilizer for fertilizing crop soils. It has a fertilizing power close to hen manure known as an excellent supply of mineral nutrients, in compost or raw state, to agricultural soils (Penjit, 2012). Sustainable soil fertilization based on mycorrhizal inoculation is also a particularly important alternative for agriculture and helps to clean up the natural environment (Tremier *et al.*, 2002). The cultivation of leguminous species is rooted in the cultural habits of peasant farmers in the tropics and subtropics. They are very advantageous for increasing and diversifying agricultural products (Megueni *et al.*, 2011). Cultivated in association with cereals in agricultural systems, they give good results compared to yields from monocultures (Tchuenteu *et al.*, 2013). It allows small agro-producers to reduce the risk of failure that can occur in monoculture. Legumes have a role in fixing atmospheric nitrogen and in particular increasing the nitrogen content of agricultural land (Agossou, 2012; Derogoh *et al.*, 2018). The high level of protein in the seeds of some food legumes makes them a recommended food for protein nutrient deficiencies. The objective of this work is to assess the impact of the application of *J. curcas* seed cake on legumes growth and seed yields as well the physicochemistry of cultivation soils.

MATERIAL AND METHODS

Description of the study site: The experiment was carried out in the field in Laï, the capital of the province of Tandjile located in the southwest of Chad. It is located in the vast area of Logone and characterized by a long dry season (7 months) and a short rainy season (5 months). Much of the province is subject to flooding and roads are impassable from August to October (Anonyme, 2011a; Kabé *et al.*, 2019). There is a diversity of soils but the sandy clay soil, suitable for agricultural and pastoral activities, is dominant (Anonyme, 2011b). The variation in rainfall is between 800 mm and 1200 mm. The maximum air humidity (80%) fluctuates between August-September. The average temperature maxima (over 40 ° C sometimes) are recorded between March and April. The average minimum temperature (15 ° C) is between December and January (Kabé, 2020).

Biological material: The seedcakes and seedpowders used in this experiment are obtained from *Jatropha* seeds cultivated in the field under various well-defined experimental conditions. *Jatropha* plants grown from pockets amended with mycorrhizal inoculum, compost and chemical fertilizer, three years old (Kabé *et al.*, 2019). The seeds of the food legume species used are those popularized by the Chadian Institute for Agronomic Research and Development (ITRAD) and the National Office for Rural Development (ONDR) whose relay is currently

provided by the National Agency for Rural Development (ANADER) in the Sudano-Sahelian zones of Chad. These are bambara groundnut (*Vigna subterranea*) and peanuts (*Arachis hypogaea*), shown (Figure 1). The groundnut is of the VTE Fleur 11 variety. It has an erect habit, a development cycle of 85 to 90 days and an estimated annual yield per hectare of between 1-5 tonnes per hectare. Bambara groundnut has a yield per hectare which can reach 0.5-1 tonnes. It is of the Djar variety with an erect habit and a 90 day development cycle. The choice of varieties of these legumes is motivated by their short life cycle and their good seed yield. The characteristics of these food legumes correspond to an adaptation in the Sudano-Sahelian zone. Note that the use of food crops with a short reproductive cycle has an advantage for the farmer who can have several harvests per year if he uses off-season cultivation methods. The experimental cultivation site is located on marginal soil, not very suitable for agriculture. This choice was made with the intention of seeing the contribution of legumes and the treatments made to the variations in the mineral levels of the soils at the end of the tests. After clearing and cleaning the experimental site, the soil is plowed by the harness oxen. The plots are maintained by manual weeding. The experimental site was protected by barriers protecting plants from herbivorous animals.

Obtaining of seedcakes and powders from the seeds of *Jatropha*: *Jatropha* for which a mixture of seeds was used in this experiment are cultivated in the field under the various experimental conditions. They are three years old in cultivation under treatment with mycorrhizal inoculum, compost and chemical fertilizer (Kabé *et al.*, 2019). Ripe fruit is picked directly from the tree. They are dried in the sun until they have a constant mass and then pulped. The black seeds removed from the fruit are dried in the sun so that they have maximum moisture loss. After removing the shells, the almonds were ground in a mechanical grinder. The paste was transferred to a cloth bag, and the whole pressed using a power press. This method allows only up to 60-70% oil to be extracted (Gbetoho *et al.*, 2016). The resulting oil was decanted, filtered and stored for possible use. The seedcakes were stocked for next use. For the seed powders, these were directly crushed in a mechanical grinder mill and then applied in the experimental plots.

Determination of climatic characteristics: The climatological data were collected on the installations of the local meteorological service of Laï. The readings were carried out in the mornings, at noon and in the evenings. These data were compared with the basic data of the General Direction of National Meteorology (DGMN) and made available by the Direction of Operation and Meteorological Applications (DEAM) through the Division of Climatology (DC).

Determination of the physicochemical characteristics of the study site soil: The determination of the sand, silt and clay content of the sites was carried out on a principle and technique based on the law of Stockes (Anonyme, 2012). Variables such as pH, conductivity, organic matter and carbon content were assessed according to the sheets set up by the Chadian Institute of Agronomic Research for Development. The Palintest System 5000 photometer used by ITRAD was used to determine the content of various minerals in the soil at the test site. It is a precision colorimeter with wide application in color matching and analytical chemistry. It therefore offers an instrumental analysis method for a wide range of water and soil tests (Anonyme, 2012).

Dependent variables and sampling size: The studies were carried out on dry biomass, Carbon stock, number of nodules, number of pods per plant, rate of attacked pods compared to healthy pods and seed yield. Ten plants were sampled per unit plot, or forty plants per treatment. The survey rate is a quotient obtained from the number of seedlings in the pockets divided by the total number of seeds placed in the pockets multiplied by one hundred (100). Dry biomass is determined at harvest. The unearthed plants were packed in labeled plastic bags and dried in the laboratory for 48 hours in an oven at 105°C. The dry weight gain or dry biomass of the plants was performed. The carbon stock is determined by multiplying the biomass value by 47.5 (% carbon in green plants) multiplied by one hundred (100) (Mugnier *et al.*, 2006). The fruit and seed yield is an estimated production per plant per hectare (ha). The nodules were counted plant by plant.

Experimental device and application of treatments: Cakes obtained from the extraction of oil by press were used. The urea was purchased from the ANADER agricultural input sales center. The experimental device consisted of 4 completely randomized blocks. The unit plots were squares with sides of 3 m (9 m²) and 0.5 m distanced one another. The blocks were separated by 1 m. The distance between the lines was 40 cm and 25 cm between the pockets (Fig. 2). The treatments were composed of: Tt (treatment consisting of Jatropha seedcake, 0.5 kg/m² or 5 tonnes/ha); Tp (consisting of powder from crushing of Jatropha seeds, 0.25 kg/m² or 2.5 tonnes/ha); Tu (Urea, 5g/m² or 50 kg/ha) and T0 (Control, represented by plants without treatment). The treatments were applied to the plants on the date of 100% flowering, corresponding to the moment when the gynophores emerged and the beginning of the formation of a bead at their end which will give pods (35 JAS on average).

Statistical analysis: The results were statistically analyzed using the Statgraphic plus version 5.0 program. Multiple analysis of variance was performed to identify the effect of different factors and blocks on the parameters studied. Duncan's test was applied for a multiple classification of the means of the different treatments. Pearson's correlation test was used to study the relationship between different dependent variables.

RESULTS

Climatic characteristics: The climatic data obtained represent the monthly averages (Table 1). The temperature peaks in March-April-May when the thermometer sometimes marks values exceeding 41°C. The minimum temperature is recorded (15°C) in the months of December-January. The annual average temperature is 28.54°C. It is generally warm throughout the year. The cumulative rainfall data recorded in two localities in the study area varied from 862.78 to 1074.4 mm of rain. The cumulative rainfall in the study area no longer reaches 1200 mm as indicated in the official documents of the Chadian state for several years according to the official figures provided by the meteorological stations of the province of Tandjilé and the General Directorate of National Meteorology (DGMN).

Physicochemical characteristics of the study site soil: The soil at the study site has an average sand content approaching 90%, about 10% clay, and just over 2% silt. It has a sandy clay texture (Table 2). The soil pH is close to neutral set at 7.

The organic matter and carbon content is relatively very low, less than 1. The values of mineral nutrients are in a moderately acceptable range. The treatments provided have substantially modified the physicochemistry of the soil. Adding the cake and powder from the seeds of *J. curcas* increased the mineral content and the soil pH. The pH, from 6,64 before the treatments, varied from 5,73 to 7,68 after the treatments. The content of organic matter and silt, respectively 0,05% and 2,02%, was increased by more than 10%.

Effect of treatments on the development and production of legumes: The contribution of cakes, crushed seeds of the *Jatropha* improves the biomass and the carbon stock of plants for the two species of these legumes (Table 3). However, there was not a significant difference between the treatments in the number of nodules.

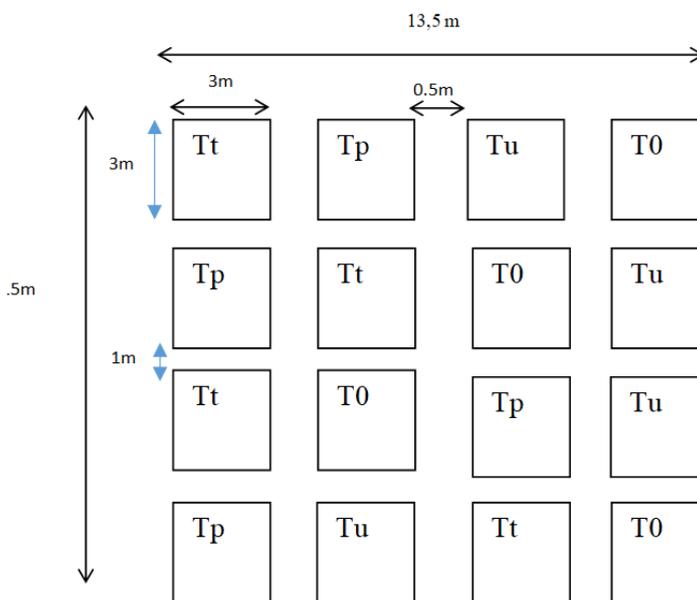
Impact of treatments on pod production and seed yield: The biocidal effects of *Jatropha* seed cakes were observed on fruit production and seed yield of peanuts and bambara groundnuts (Table 4). There is a significant difference at the 5% threshold between the treatments applied. The number of healthy pods per plant and the yield of two food legumes seeds were greatly increased. Application of these products helped protect the pods from attack by peanut and bambara groundnuts pests such as centipedes (Table 4a and 4b). The attacked pods are more numerous on untreated or control plots. The urea allowed a good filling of the hulls hence the high weight of the seeds per hectare. However, its contribution causes the acidity of the soil and creates an environment hostile to the life of the microfauna and the microflora of the soil. In addition, the delay in maturation of plants on plots treated with urea is observed. The low seed yield compared to forecast is due to poor soil (Table 1).

DISCUSSION

The climate is an important explanatory factor of agricultural production. Thus, very or not abundant rainfall can hamper the development of plants, thereby limiting annual agricultural productivity (Derogoh *et al.*, 2018). The climatic hazards noted everywhere at the moment due to climate change are strongly felt on the spot. The maximum annual rainfall of 1200 mm has not been reached for a few years. The beginning of the rainy season announced in the screen in the months of March-April takes place in the month of May. Maximum air humidity (70% on average) was reached in August-September, instead of 80% other time (Kabe *et al.*, 2020). The variations in rainfall recorded could also induce changes in the soil level of the province (Abdoulaye, 2018). Planting trees and using cover crops could combat climate change and stabilize agricultural soils (Ngakou *et al.*, 2012; Tchobsala *et al.*, 2013). Edaphic factors are the main elements influencing the growth, development and production of plants grown in fields. In the tropics and subtropics the knowledge of soil variation and its nutrient content must be mastered by farmers. Soil structure has a major influence on water retention, air circulation, biological soil activity, root growth and seedling emergence (Ab van, 2010; Kabe *et al.*, 2019). The granulometry and physicochemistry of the soil studied globally prove that it can be used for agricultural activities (Anonyme, 2012). Indeed the low carbon content and the material could compromise a good production. It would therefore be necessary to add fertilizers, in particular an amendment of organic matter to boost the level of nutrients and increase the biological activities of the soil (Laba *et al.*, 2017; Rediné *et al.*, 2019).



Figure 1. Seeds of edible legumes popularized by ITRAD and ONDR in the Sudano-Sahelian zones of Chad, A. Bambara groundnut. B. Peanut



Tt : treatment consisting of *Jatropha* seedcakes; Tp: treatment consisting of *Jatropha* seedpowders; Tu: treatment consisting of Urea; T0 : control

Figure 2. Experimental design for biocidal trials of *Jatropha* on legume pests

Table 1. Climatic factors

Climatic parameters	Jan.	Feb.	Marc	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Temperature (°C)	23,65	27,8	32	33,25	33,45	30	27,6	26,75	27	27,7	27,25	26,05
Rainfall(mm)	0	0	0	25,3	95,3	243,2	137,4	284,8	277,3	34,5	0	0

Table 02. Particle size and physicochemical characteristics of soil at study site

Studied parameters	Average values before treatments	Average values after treatments			
		Tt	Tp	Tu	To
Sand (%)	88,19	79,85	79,03	87,11	88,01
Silt (%)	2,02	10,12	10,04	3,21	2,23
Clay (%)	9,79	10,03	10,93	9,68	9,76
Water pH	6,64	7,38	7,68	5,73	6,24
Conductivity (µs/cm)	20,46	332	345	50,32	30,54
Organic material(%)	0,05	10,31	11,84	1,82	2,02
Carbon (%)	0,03	7,18	7,46	1,12	1,82
N total (ppm)	35,75	66,21	63,47	81,12	39,75
P total (ppm)	255	340,54	341,10	331,58	265,12
K total (ppm)	157,5	250,10	261,86	202,66	176,35
Mg (ppm)	47,21	71,39	70,44	60,68	50,23
Ca (ppm)	260,30	370,25	330,27	226,52	270,31
Fe (ppm)	776,11	1008,12	1010,14	778,41	772,17
Cu (ppm)	85,52	88,56	86,87	83,94	87,54
Mn (ppm)	20,23	25,10	31,01	21,22	22,51
S (ppm)	21,22	32,41	41,31	22,22	23,42
Zn (ppm)	15,32	34,12	35,51	18,32	18,62

Tt : treatment consisting of *Jatropha* seedcakes; Tp : treatment consisting of *Jatropha* seedpowders; Tu : treatment consisting of Urea; T0 : control

Table 3a. Impact of treatments on biomass, carbon stock, number of nodules in peanuts (*Arachis hypogaeae*)

Treatments	Dry biomass (g/plants)	Carbon stock (Kg/ha)	Number of nodules
Seedcake	181,11±0,07 ^b	86,02±2,02 ^b	33,32±0,83 ^a
Crushed Seeds	176,32±0,05 ^b	83,75±2,21 ^b	31,31±0,33 ^a
Ureas	198,24±0,01 ^c	94,16±1,17 ^c	30,65±0,34 ^a
Control	158,82±0,04 ^a	75,43±3,11 ^a	33,32±0,83 ^a
P	0,031	0,012	0,121

Values in the same column by the same letter are not significantly different (P<0,05).

Table 3b. Impact of treatments on biomass, Carbon stock, number of nodules in bambara groundnut (*Vigna subterranea*)

Treatments	Dry biomass (g/plants)	Carbon stock (Kg/ha)	Number of nodules
Seedcake	222,23±0,02 ^b	105,56±2,02 ^b	32,26±0,25 ^a
Crushed Seeds	214,24±0,04 ^b	101,54±2,21 ^b	32,12±0,29 ^a
Ureas	272,56±0,04 ^c	129,46±1,17 ^b	29,13±0,12 ^a
Control	202,13±0,01 ^a	96,01±3,11 ^a	31,16±0,25 ^a
P	0,011	0,022	0,321

Values in the same column followed by the same letter are not significantly different (P<0.05).

Table 4a. Biocidal effects of *Jatropha* seeds on pod production and peanut seed yield (*Arachis hypogaeae*)

Treatments	Dry biomass (g/ plants)	Rate of pods attacked	Seed yield (kg / ha)
Seedcake	42,12±9,11 ^b	1,6±1,72 ^a	702,52±9,11 ^b
Crushed Seeds	40,13±7,13 ^{ab}	2,13±1,76 ^a	710,51±9,21 ^b
Ureas	40,73±8,58 ^{ab}	2,33±3,33 ^a	1125,23±8,02 ^c
Control	31,33±9,81 ^a	18,21±1,22 ^b	405,53±5,23 ^a
P	0,0036	0,012	0,021

Values in the same column followed by the same letter are not significantly different (P<0.05).

Table 4b. Biocidal effects of *Jatropha* seeds on pod production and seed yield of bambaragroundnut (*Vigna subterranea*)

Treatments	Dry biomass (g/ plants)	Rate of pods attacked	Seed yield (kg /ha)
Seedcake	37,32±0,32 ^b	3,21±1,22 ^a	587,22±0,23 ^b
Crushed Seeds	38,21±0,41 ^b	1,11±1,31 ^a	618,17±0,27 ^c
Ureas	48,42±1,23 ^c	2,83±1,13 ^a	807,14±0,23 ^d
Control	32,51±0,33 ^a	22,34±1,21 ^b	537,41±0,33 ^a
P	0,0021	0,032	0,01

Values in the same column followed by the same letter are not significantly different (P<0.05).

Soil is subjected to a number of chemical reactions, both organic and inorganic. These chemical reactions define the soil pH which is an important factor in the release of nutrients into the soil. When the pH is below 5.5, many of the nutrients are soluble and available for uptake by plant roots. At high pH, nutrients become insoluble and can no longer be absorbed (Ab van, 2010; Goumong *et al.*, 2017). The maximum fertility of agricultural soil corresponds to a pH close to neutrality (6,59 < pH< 7,38) to provide substantial crop production. This is where most nutrients are absorbed (Ab van, 2010; Kanabo and Gilkes, 1987). In this study, the contribution of meal and powder from the seeds of *J. curcas* constitutes an organic amendment that enhances the sustainable development of agriculture without the use of chemical fertilizers (Nwaga, 2009). Sterile soils end up revitalizing. The proportions of soil minerals have been greatly increased. The pH, which tends towards neutrality, could increase the availability of minerals that can be absorbed by the roots of plants. This could explain the improvement in biomass and the carbon stock of plants. It means that there is a good sequestration of carbon from the air by plants, therefore a decrease in CO₂ contributing to the fight against change climate caused mainly by this gas which causes global warming. However, the pH on the plots treated with urea decreases. According to Ngakou (2012), the intensive application of chemical fertilizers acidifies agricultural soils and can interfere with the soil microflora. The cakes and powder of the seeds of *J. curcas*, in addition to its effect of protecting plants by its insecticidal activity, provides nitrogen,

phosphorus, potassium and other minerals essential to the soil for the good development of the plant (Marion, 2013; Minengu 2014). It is an excellent organic fertilizer to promote organic farming, especially thanks to its high content of nitrogen, phosphorus, potassium (NPK). The mineral content and fertilizing power of *J. curcas* cakes are equivalent to the mineral content of domestic poultry droppings (Jongschaap, 2007; Biekre *et al.*, 2018). The digestate obtained after bio-digestion of *J. curcas* seed cakes to produce the biogas contains 5.56% nitrogen, 2.90% phosphorus and 1.24% potassium and is a good fertilizer due to its nutrient composition (Ali *et al.*, 2010). Reinhard (2005) reported that almost one tonne of *Jatropha curcas* meal is equivalent to 200 kg of mineral fertilizer. Application can be coarse or mixed with animal droppings and crop residues such as straw from rice cultivation (Das *et al.*, 2010). These organic fertilizers make it possible to limit losses by leaching nitrogen and potassium (Domergue and Pirot, 2008). But at high doses, greater than 5 tonnes/ha, *J. curcas* seed cakes has phytotoxic effects during the germination stage (Marion, 2013). The phytosanitary protection of legumes is ensured by the biocidal potential of *J. curcas*, namely toxic substances, namely phorbol esters, curcin as well as other insecticidal and insect repellent compounds (Marion, 2013). The molluscicidal and larvicidal activities from preparations based on the seeds of *J. curcas* have been reported (Rug *et al.*, 2000). These are well-known practices of organic farmers (Nwaga, 2009; Pina *et al.*, 2005).

The use of *J. curcas* meal in agricultural production could entail the risk of transfer of phorbol esters and other toxins remaining very active in the meal to cultivated edible plants. Devappa *et al.* (2010) hypothesized that the main toxic compounds present in *Jatropha* seed cakes must be found in the soil, but their own work in the field showed that the phorbol esters contained in the oil of *J. curcas* are completely degraded after 12 to 19 days at room temperature. On the other hand, Penjit *et al.* (2012) proved that the phorbol esters contained in the cakes are degraded in the soil after 17 to 21 days under the same conditions. These observations suggest that fungi could be used to facilitate the degradation of certain toxic compounds. Indeed, Belewu *et al.* (2010), through fungal fermentation, reduced the amount of phorbol esters by approximately 77% and the saponin content by nearly 95% in cakes inoculated with *Aspergillus niger*.

The degradation products obtained seem less toxic since they have no effect on molluscs (living organisms very sensitive to low doses of phorbol esters). In addition, it has been proven that phorbol esters, pure or present in a meal, were completely biodegraded in the soil, in about twenty days at 23°C and in less than 15 days above 32°C. This degradation appears to be favored by soil moisture (Kasuya *et al.*, 2013). A plausible study on the non-toxicity of agricultural products at harvest was carried out on three plant species, the different parts of which enter the human diet. These were Chinese cabbage for the leaves, tomato for the fruit and sweet potato for the tubers. This study proved that phorbol esters, the most dangerous of the toxins in *Jatropha* seeds, are not found either in the consumable parts of plants, or in the soil. The use of *Jatropha* cake therefore does not lead to the accumulation of toxic molecules in plants and in the soil (Penjit *et al.*, 2012). The seed cakes have also been used to control nematodes in tomato crops (Devappa *et al.*, 2010).

CONCLUSION

The use of *Jatropha curcas* seed cakes, crushed seeds increases the biomass, the number of pods per plant and the seeds yield of peanut and bambara groundnut. The biocidal potential possessed by *J. curcas* allows protection against pest insects and to obtain a high number of healthy pods thanks to the toxic substances with insecticidal and insect repellent effect in the seeds of *J. curcas*. Urea allows a good filling of hulls where the high weight of seeds per hectare, however, its contribution acidifies the soil. The degradation of *J. curcas* toxins after three weeks leads to its non-accumulation in the vegetable matter to be consumed. The contribution of *J. curcas* cakes allows, in addition to the phytosanitary protection of crops intended for human consumption, the fertilization of the soil with organic matter. It bodes well for a better future in promoting the practice of organic farming, without the use of chemical fertilizers.

Conflict of interest statement: Authors declare that they have no conflict of interest.

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