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SEED CAKE OF *JATROPHA CURCAS* (L.), POTENTIAL SUBSTRATE TO PRODUCE MAGGOTS AS FEED FOR REARED MONOGASTRIC ANIMALS

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ABSTRACT

Seeds of jatropha (*Jatropha curcas*) are used worldwide to produce biodiesel and the oil extraction generates a high amount of waste called seed cake. Jatropha seed cake has high protein content but its valorization, for example as animal feed, is hampered by its toxicity and anti–nutritional properties. This study, carried out in Benin, West Africa, aimed to determine the capacity of jatropha seed cake to produce fly larvae for animal feed and assess the nutritional quality of the larvae. Three types of substrates were exposed to naturally occurring house flies: jatropha seed cake alone, the same seed cake enriched with earthworms to help detoxifying and decomposing the cake, and corn bran, a substrate commonly used in the region to produce fly larvae. All three substrates produced larvae of similar sizes. The yield obtained with corn bran was significantly higher (55.6 g per kg of dry substrate) than those with pure seed cake (44.5 g) and seed cake with earthworms (50.1 g). However, the larvae produced with jatropha seed cake contained more proteins and other important nutrients than those produced with corn bran. These results show that jatropha seed cake can be valorized by producing fly larvae but it remains to be seen whether the fly larvae produced on jatropha seed cake are not toxic for animals.

Key words: biomass maggot, Maggots size, earthworms, *substrate*, chemical composition.

INTRODUCTION

Jatropha (*Jatropha curcas* (L.)) is a biofuel crop planted worldwide in tropical climates to produce biodiesel and, secondarily, other products such as soap (Achten *et al*., 2008; Henning 2008; Gbemavo, 2014). In Africa, it is most suitable when produced in a family labour setting (van Eijck *et al*., 2012) and when by-products are valorized to their maximum (Muys *et al*. 2014). One of these by-products is the seed cake that is produced when the seeds are pressed for oil extraction. This cake contains minerals and proteins and can be utilised as organic fertiliser (Francis *et al*., 2005) but its use as animal feed is hampered by the fact that it also contains curcin, a toxic lectin, and anti-nutritional factors (Openshaw, 2000; Kasuya *et al*., 2012; Gogoi *et al*., 2015). The seed cake may be disposed improperly, causing concern for potential environmental damages. Thus, there is a need to find other valuable uses for the seed cake.

One solution could be to use jatropha seed cake to produce fly larvae for animal feed. Fly larvae are easy to produce on animal and plant waste and can be incorporated as protein source in the diet of monogastric animals such as poultry and fish (Kenis *et al*. 2014). Fly larvae are already naturally consumed by scavenging poultry (Smith, 1992) and, in Benin up to 6% of the farmers use them regularly to feed their poultry (Pomalégni *et al*. 2016). As elsewhere in Africa, traditional poultry production faces major constraints related to the lack of feed resources especially proteins (Hardouin, 1986; Sonaiya and Swan 2004; Pousga *et al*. 2005; Ayssiwede *et al*. 2011). The two fly species most commonly produced for animal feed are the black soldier fly, *Hermetia illucens*, and the house fly, *Musca domestica* (Pastor *et al*. 2015). There are several methods to produce fly larvae but an easy one consists in exposing suitable and attractive substrates and allow flies to oviposit naturally (Kenis *et al*. 2014). This method is already used in Africa for producing house flies (Koné *et al*. 2017). Jatropha seed cake have high protein content and could be very suitable for fly larvae production.

However, the larvae production could be hampered by the toxicity of the seed cake. Earthworms are recognized as bio-indicators of soil quality because they are susceptible to contamination by metal trace elements and these elements accumulate in their tissues (Beaumelle, 2015). The use of earthworms in combination with seed cake of *J. curcas* could help detoxify the cake and facilitate its decomposition.

In this study, we assess the suitability of jatropha seed cake, with and without earthworms, for house fly larvae production that can be used in the feeding of local chickens.

MATERIALS AND METHODS

Study area: The experiment was conducted at the site of Sub-Programm of unconventional animal species breeding (SPEEANC) of the National Institute of Agricultural Research of Benin (INRAB). The SPEEANC is situated at 15 km north-west of Cotonou, Benin in a region characterized by a climate of Guinean type with two rainy seasons (mid-March to mid-July and mid-September to mid-November), two dry seasons (mid-November to mid-March and mid-July to mid-September). The average annual rainfall of the site is around 1,200 mm, 700 mm to 800 mm for the major rainy season and 400 to 500 mm for the minor rains. The monthly average temperature ranges between 27 and 31 °C with a deviation of 3.2 °C between the hottest month (March) and the least hot month (August) (Ogou, 2009).

Effect of the substrate on the biomass and maggot size: The experimental design was a Complete Random Design (CRD) with 10 repetitions. The studied factors were the substrate type (pure seed cake of jatropha; seed cake of jatropha supplemented with 10 live earthworms (*Eisenia foetida*); and corn bran) and ten levels block factor. Corn bran was used as a control suitable substrate for fly larvae rearing (Mensah *et al*. 2007; Pomalégni *et al*. unpublished). Each experimental unit was represented by an open plastic basin containing 1 kg of each type of substrate. In total, thirty experimental units were considered. The substrates were moistened to saturation (1L of water for 1kg of pure seed cake of *J. curcas* and 1.5L of water for 1kg of corn bran), then covered with a thin cloth for three days to cause fermentation. This cloth was also used to avoid fly oviposition during the fermentation process. After three days, the fermented substrates were exposed to naturally occurring flies (mostly *Musca domestica*) for 24 hours to enhance oviposition. Maggots were harvested at maturation, i.e. four days after exposure, using a two-mesh sieve. Fresh maggots' biomass (g) of each experimental unit was quantified using an electronic scale with 1g accuracy. The maggot size (in mm) was measured on 100 maggot sample randomly chosen from each experimental unit using a double decimeter (graduated in millimeters). Live earthworms were first used for detoxification and cake decomposition (Sharma *et al*. 2009) but since they died within the three days of fermentation, their cadavers probably functioned as attractant for the ovipositing flies.

Effect of the substrate on the chemical composition of maggots: The nutritional quality of maggots obtained from each type of substrate was evaluated by determining the proximal physico-chemical composition. For this, all replicates from each type of substrate were carefully mixed and a sample of two hundred grams (200g) of maggots from each substrate was used for the evaluation of their contents in Dry matter (DM) basis. The rate of nitrogen (N) was determined by the Kjeldahl (block digestion) method (AOAC 981.10) (AOAC, 1990) and Raw ash was obtained by calcination at 550°C. Total Phosphorus (P) was determinedby the colorimetric method (Bray and Kurtz, 1945), Potassium (K) by Atomic Absorption Spectrophotometer (Sahrawat *et al*., 2002; Wani *et al*., 2012). Samples were digested with concentrated HNO_3–H_2O_2 at 120°C during 3 hours in a Teflon digester. Digested samples were thereafter diluted with distilled water. Total Na, Mg and Ca. contents were measured by atomic absorption spectrophotometry using an AAS-800 spectrophotometer (Perkin Elmer, Wellesley, MA, USA). Total N content was determined by the Kjeldahl (block digestion) method (AOAC 981.10) (AOAC, 1990) and Rawash was obtained by calcination at 550°C. All analyzes were conducted at the Lab of Soil Science, Water and Environment (LSSEE) of the National Institute of Agricultural Research of Benin (INRAB).

Statistical data processing: The effect of different factors (substrate and block) on biomass maggots was examined using a linear mixed effects model (Pinheiro and Bates, 2000). In this model, the fixed effect was the substrate while the random effect was the block. The existence of the effect of blocks in the data had been previously tested by empty models (*unconditional means model*: Singer and Willett, 2003). From the statistical model, the adjusted biomasses by type of substrates were estimated. The nonparametric Kruskal-Wallis test was used to compare the morphology (size) maggots between types of substrates as maggots size data were not normally distributed (Normality Test of Ryan-Joiner). The adjusted maggots' size by type of substrate was also estimated. The adjustment of generalized linear mixed effects model to the data and the Kruskal Wallis test for normality were made in the R3.2.0 software (R Development Core Team. 2012, http:// www. Rproject. org/). The lmer function of package lme4 and the lmer Test package were used for linear mixed effect model while the kruskal.test function was used for the Kruskal Wallis comparison test.

RESULTS

Effect of the substrate on the biomass and maggot size: The overall result of the mixed effects linear model revealed that the substrate had significantly influenced (F = 9.77; P=0.00134) the biomass of maggots. The block did not have a significant effect on biomass maggots. Nevertheless there was 40% variation between blocks. The biomass of maggots varied significantly between the types of substrates (Table 1). No significant difference in the size of maggots among the substrates was found (H = 3.58; P =0.1669). Table 2 shows the adjusted biomass and size of maggots produced by type of substrate. The biomass and size of maggots obtained from corn bran are higher than those recorded in the other substrates. Furthermore, the average estimated biomass of maggot produced by the composite substrate seed cake of *J. curcas* with earthworms was significantly greater than that produced by the pure seed cake of *J. curcas*.

Table 1. Effect of the substrate on biomass maggots: detailed results of the linear mixed effects model.

Sources de variation	Estimation	Standard Error type	Signif. t-value
Intercept	55,6	2.275	0,000
Pure seed cake of *Jatrophacurcas*	-11,1	2,511	0,000
Seed cake of *Jatrophacurcas* with earthworms	-5,5	2,511	0.0418

Table 2. Average of the biomass and size of maggots.

Substrate	Size (cm)	
Corn bran	55.60±2.27	1.093±0.01
Pure seed cake of *Jatrophacurcas*	44.50±2.27	1.062±0.01
Seed cake of *Jatrophacurcas* with earthworms	50.10±2.27	1.061±0.01

± = Standard Error

Effect of the substrate on the chemical composition of maggots: The results of the chemical analysis of maggots produced are presented in Table 3. The highest concentrations of all studied minerals were found in the maggots that were produced with substrates of seed cake of *J. curcas* and earthworms, except the nitrogen that was higher in pure seed cake *of J. curcas*, which also contained the highest amount of protein.

Table 3.Chemical composition (% DM) of maggots produced from seed cake of *Jatropha curcas* and Corn bran.

Chemicalparameters	Maggots from substrate		
	Corn bran	**Seed cake of *J. curcas***	**Seed cake of *J. curcas* with earthworms**
Dry matter	31.071	30.837	**32.274**
Sodium	0.141	0.155	**0.157**
Calcium	0.135	0.704	**0.880**
Magnesium	0.494	4.009	**4.640**
Potassium	0.912	1.325	**1.469**
Phosphorus)	0.927	3.852	**4.289**
Nitrogen	7.034	**7.728**	6.877
Protein	43.960	**48.300**	42.980
Crudeash	4.872	18.323	**21.179**

DISCUSSION

Effect of the type of substrate on the biomass of maggots: While no significant difference was found among substrates in the size of maggots, the average amount of maggots produced in four days with corn bran was significantly higher than that obtained with the seed cake of *J. curcas* supplemented with earthworms and pure seed cake of *J. curcas*. There are many kinds of substrates, of animal or plant origin, that can be used to produce flies larvae, varying according to agro-ecological zones, regions and countries (Bouafou *et al*., 2006; Pomalégni *et al*., 2016; Koné, 2017). The yields obtained in this study with corn bran and jatropha seed cake, i.e. 44-56 g of fresh fly larvae per kg of dry substrate, are rather low compared to those obtained in optimized systems (e.g. annual average of 124 g of larvae per kg of dry chicken manure, Koné *et al*. 2017) but others report much lower yields with other substrates (Ekoue and Hadzi 2000; Teguia *et al*. 2002). Yield can vary tremendously with substrates but also with seasons and locations (Bouafou *et al*. 2006; Koné *et al*. 2017). Our own studies (Pomalégni *et al*., unpublished) have shown that fermented corn bran often produce much higher yields (>100g of larvae per kg of dry bran) in other circumstances. Thus, corn bran is a good standard substrate against which other substrates can be compared. Thus, the fact that the amount of biomass produced by jatropha seed cake is rather similar to corn bran is promising. Furthermore, no dead larvae were found in the jatropha seed cake, suggesting that fly larvae can overcome the toxicity of curcin. Nevertheless, considering that jatropha seed cake is much richer in proteins (50-64%, Gogoi *et al*. 2015) than corn bran (ca. 12%, Heuzé *et al*. 2015) and that proteinic substrates favour the development of fly larvae (Bouafou *et al*. 2006), a higher yield could have been expected. But the chemical content of the substrates is not the only criterion for a good yield. The physical structure of the substrate is also essential to allow a high oviposition rate and a good development of the larvae (Kone *et al*. 2017) and, in this respect, the jatropha seed cake, as provided in this experiments, may be less favorable that corn bran. However, the physical structure of a substrate can be easily improved, e.g. by adding cellulose to enhance aeration. Jatropha seed cake is also characterized by a slow degradation that may hamper a proper assimilation by the maggots.

Role of earthworms in the substrate: The primary objective of the introduction of earthworms in the seed cake of *J. curcas* was to detoxify the cake and facilitate its decomposition. However, the earthworms quickly died in the cake and the cadavers were thus probably used by the flies as attractants, leading to the production of a higher biomass of larvae compared to pure seed cake. It is a well-known fact that adding a small amount of highly proteinic attractants of animal origin, such as blood or fish offal boosts the productivity of a substrate for fly production (Nzamujo, 1999; Bouafou *et al*., 2006; Mensah *et al*., 2007; Koné *et al*. 2017). Thus, while earthworms cannot be recommended as additive to jatropha seed cake, adding other animal waste could be considered to maximize larvae production.

Influence of the substrate on the chemical composition of maggots: The chemical composition of maggots is influenced by the composition of the substrate on which they fed (Makkar *et al*., 2014; Charlton *et al*., 2015; Tschirner and Simon, 2015). Our analyzes showed that maggots produced from jatropha seed cake are richer in proteins and several nutrients than maggots from corn bran. This can be explained by the fact that jatropha seed cake is richer in proteins and various nutrients than corn bran (Gogoi *et al*. 2015; Heuzé *et al*. 2015). The protein content of maggots from corn bran (44%) was unusually low for house fly (Makkar *et al*. 2014; Pastor *et al*. 2015) whereas the content obtained in maggots produced from seed cake (48.3% DM) was close to the average (50.4% DM) calculated by Makkar *et al.* (2014).

Conclusion: Seed cake of *J. curcas* is a by-product obtained after extraction of the oil from seed. Presently, the only obvious way to use it is as organic fertilizer in the fields. The present study shows that it has the potential of being first used to produce fly larvae before being applied in the fields. This would allow, firstly, to produce a valuable source of monogastric animal feed and, secondly, to initiate the process of decomposition for the assimilation of nutrients by crops. Indeed, residues of other fly larvae production systems produce highly valued composts (Nzamujo, 1999; Koné *et al*. 2017). Fly larvae yields produced in this study are satisfactory but could be largely improved through the addition of an attractant for female flies or modification of the physical structure of the seed cake. However, it remains to be seen whether the fly larvae produced on jatropha seed cake are suitable for feeding animals and do not contain toxic and anti-nutritional factors contained in the seed cake of *J. curcas*.

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