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Detection of swainsonine and calystegines in Convolvulaceae species from the semiarid region of Pernambuco[1]

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ABSTRACT. - Mendonça F.S., Silva Filho G.B., Chaves H.A.S., Aires L.D.A., Braga T.C., Gardner D.R., Cook D. & Buril M.T. 2018. [**Detection of swainsonine and calystegines in Convolvulaceae species from the semiarid region of Pernambuco].** *Detecção de swainsonina e calisteginas em espécies de Convolvulaceae da região semiárida de Pernambuco. Pesquisa Veterinária Brasileira 38(11):2044-2051.* Laboratório de Diagnóstico Animal, Departamento de Morfologia e Fisiologia Animal, Universidade Federal Rural de Pernambuco, Rua Dom Manoel de Medeiros s/n, Dois Irmãos, Recife, PE 52171-900, Brazil. E-mail: fabio.mendonca@pq.cnpq.br

Numerous plant species worldwide including some *Ipomoea* (Convolvulaceae) and *Sida* (Malvaceae) species in Brazil cause lysosomal storage disease in herbivores and are known to contain swainsonine and calystegines as the main toxic compounds. The aim of this work was to determine swainsonine and calystegines concentrations in species of Convolvulaceae from the semiarid region of Pernambuco. Seven municipalities in the Moxotó region were visited and nine species were collected and screened for the presence of swainsonine and calystegines using an HPLC-APCI-MS method. The presence and concentration of these alkaloids within the same and in different species were very variable. Seven species are newly reported here containing swainsonine and/or calystegines. *Ipomoea subincana* contained just swainsonine. *Ipomoea megapotamica*, *I. rosea* and *Jacquemontia corymbulosa* contained swainsonine and calystegines. *Ipomoea sericosepala*, *I. brasiliana*, *I. nil*, *I. bahiensis* and *I. incarnata* contained just calystegines. The discovery of six *Ipomoea* species and one *Jacquemontia* species containing toxic polyhydroxy alkaloids reinforces the importance of this group of poisonous plants to ruminants and horses in the semiarid region of Pernambuco. Epidemiological surveys should be conducted to investigate the occurrence of lysosomal storage disease associated to these new species.

INDEX TERMS: Poisonous plant, swainsonine, calystegines, Convolvulaceae species, Pernambuco, alkaloids, lysosomal storage disease, plant poisoning, herbivores, toxicoses.

RESUMO.- [**Detecção de swainsonina e calisteginas em espécies de Convolvulaceae da região semiárida de Pernambuco.**] Numerosas espécies de plantas em todo o mundo, incluindo algumas espécies de *Ipomoea* (Convolvulaceae) e *Sida* (Malvaceae) no Brasil, causam doença de armazenamento lisossomal em herbívoros e são conhecidas por conterem swainsonina e calisteginas como princípios tóxicos. O objetivo deste trabalho foi determinar a concentração de swainsonina e calisteginas em espécies de Convolvulaceae da região semiárida de Pernambuco. Sete municípios na região do Sertão do Moxotó foram visitados, onde foram coletadas amostras das folhas de nove espécies de Convolvulaceae para avaliação da presença de swainsonina e calisteginas utilizando-se cromatografia líquida com espectrometria de massa. A presença e concentração destes alcaloides nas folhas de plantas da mesma espécie e dentre as espécies foram muito variáveis. Seis novas espécies de *Ipomoea* e uma espécie de *Jacquemontia* contendo swainsonina e/ou calisteginas são relatadas neste

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estudo. *Ipomoea subincana* continha apenas swainsonina. *Ipomoea megapotamica*, *I. rosea* e *Jacquemontia corymbulosa* continham swainsonina e calisteginas. *Ipomoea sericosepala*, *I. brasiliana*, *I. nil*, *I. bahiensis* e *I. incarnata* continham apenas calisteginas. A descoberta de novas espécies de *Ipomoea* e *Jacquemontia* contendo alcaloides polihidroxílicos tóxicos reforçam a importância deste grupo de plantas tóxicas para ruminantes e equinos na região semiárida de Pernambuco. Pesquisas epidemiológicas devem ser realizadas para investigar a ocorrência de doença de depósito lisossomal associada a essas novas espécies.

TERMOS DE INDEXAÇÃO: Plantas tóxicas, swainsonina, calisteginas, Convolvulaceae, Pernambuco, alcaloides, doença de depósito lisossomal, intoxicação por plantas, herbívoros, toxicoses.

INTRODUCTION

Swainsonine-containing plants comprises a group of several toxic weeds that impair the development of pasture and livestock worldwide. These plants belong to three genera of the Fabaceae family (*Astragalus*, *Oxytropis* e *Swainsona*) (Burrows & Tyrl 2012, Cook et al. 2009, 2014), one genus of the Convolvulaceae family (*Ipomoea*) and one genus of the Malvaceae family (*Sida*) (Oliveira Júnior et al. 2013) and are distributed mainly in the United States, Canada, Mexico, Brazil, Russia, Spain, Ireland, Morocco, Egypt, Australia and China (Jung et al. 2011, Cook et al. 2014, Oliveira Júnior et al. 2013).

Swainsonine is a polyhydroxy alkaloid, a potent inhibitor of lysosomal α-mannosidase and α-mannosidase II of Golgi apparatus of neurons and epithelial cells. Some calystegines are potent glycosidases inhibitors and is suggested to disrupt intestinal glycosidases, lysosomal function and glycoprotein processing causing enzymatic dysfunction and accumulation of complex oligosaccharides in lysosomes (Colegate et al. 1979, Dorling et al. 1980, Stegelmeier et al. 2008). Recent research has shown that swainsonine is a secondary metabolite resulting from endophytic fungi and is not therefore produced by the host plant. The presence of endophytic fungi was demonstrated in *Astragalus*, *Oxytropis*, *Swainsona* and *Ipomoea* species (Braun et al. 2003, Pryor et al. 2009, Hao et al. 2012, Cook et al. 2014). However, it is suggested that calystegines are secondary metabolites resulting from plants as it has been demonstrated in a previous study when these toxins remained present even in plants derived from seeds treated with fungicides (Cook et al. 2013).

Clinical signs presented by ruminants and horses grazing swainsonine-containing plants consists mainly in neurological disorders, although endocrine and reproductive alterations may also occur (Oliveira et al. 2011, Tokarnia et al. 2012). Neurological changes observed are mainly of cerebellar origin, evidenced by loss of balance, followed by falls when the animals are stressed, ataxia, hypermetria, dysmetria, nystagmus, lateral gait and head and neck tremors. After the falls, the animals have difficulty into raising up and may present spasticity of the hindlimbs. Other alterations, such as somnolence, progressive emaciation and bristly, brittle and opaque hair may be observed (Tokarnia et al. 2012, Mendonça et al. 2011, 2012, Lima et al. 2013, Oliveira Júnior et al. 2013, Rocha et al. 2016).

Convolvulaceae family has a wide distribution worldwide, comprises 60 genera and at least 1.900 species. *Ipomoea* and *Jacquemontia* are being the most representative genera of this family with most of their species endemic in Brazil (Staples 2012, Bianchinni & Ferreira 2013, Buril et al. 2015). During epidemiological investigations of neurological diseases in ruminants, especially goats in the semiarid region of Pernambuco, our research team has observed outbreaks of lysosomal storage disease in which several species of *Ipomoea* could be associated to the disease, some of them being known to be toxic but in another hand, most species remains unknown about their alkaloid contents and toxicity. For this reason, the aim of this study was to determine swainsonine and calystegines concentrations in species of Convolvulaceae from the semiarid region of the State of Pernambuco.

MATERIALS AND METHODS

This study was performed in the microregion of the Sertão do Moxotó, Pernambuco, in the municipalities of Arcoverde, Sertânia, Betânia, Ibimirim, Custódia, Inajá and Manari. The weather in these municipalities is semiarid, with high temperatures and scarce and badly distributed raining. The characteristic vegetation is the Brazilian Caatinga Savannah. This study was performed during the rainy season in 2017, which comprehends the months from March to June. The main highways of those municipalities and bordering roads throughout rock outcrops and livestock farms and were car-driven with an average speed of 18 mph making a minimum distance of 62 miles and a maximum of 124 miles in each municipality, to collect and determine the occurrence of Convolvulaceae species. Those species observed during the rout were collected for botanical identification and vouchers were deposited in Vasconcelos Sobrinho Herbarium of the Federal Rural University of Pernambuco. Samples containing 500g of leaves from each species (five individuals by species) were collected, dry shaded, crushed, mixed to create a pool of samples and analyzed by high-performance liquid chromatography–atmospheric pressure chemical ionization–tandem mass spectrometry (HPLC-APCI-MS) method, according to the procedures described by Gardner et al. (2001).

Samples (100g) of the dry vegetal material were used to obtain extract and posterior analysis for presence of swainsonine by ion exchange resin insulation. The aqueous extract was then analyzed by liquid chromatography coupled to mass spectrometry using a HP 1100 binary solvent pump, an automatic sampler, a HPLC column of Betasil C18 reverse phase (100x2mm) and a Finnigan LCQ mass spectrometer. Swainsonine was eluted using an isocratic mixture of 5% methanol in 20 mM ammonium acetate at a flow rate of 0.5mL/min. The size of the sample injection was 20 μL. Ionization was achieved using an atmospheric pressure chemical ionization (APCI) source with a vaporization temperature of 450°C and a corona discharge current of 5 μA. The mass spectrometer was conducted in a MS/MS mode, examining the ions of the products in a mass range of 70-300 amu after the fragmentation of the protonated swainsonine molecule using a relative collision energy setting of 25%.

The area of the swainsonine peak was measured from the reconstructed-ion chromatogram (m/z 156) and the quantification was based in an external standard calibration. The presence of swainsonine was verified through gas chromatography - mass spectrometry (GC/MS) after a portion from the aqueous extract was dried and the residue derived by the addition of N,O-bis (trimethylsilyl) trifluoroacetamide (BSTFA) and pyridine to convert swainsonine into its timesilil ether derivative. GC/MS analysis was performed using an electronic therm GC/MS system installed with an Agilent DB-5MS capillary column (30m×0.25mm). Helium was used as a carrier gas

at a constant flow rate of 1.5 mL/min. The samples (2.0µL) were injected using a split/splitless injector with a temperature of 250 °C. The column temperature was programmed at 120°C for 1 min, increased from 120 to 200°C at 5°C/min and from 200 to 300°C at 20°C/min and then maintained at 300°C for 8 min. The presence of calystegines was determined using the same GC/MS data to verify the existence of swainsonine.

The plants were photographed and the data on their occurrence were compared with the pluviometric indexes of each municipality, according to data from the Instituto Agronômico de Pernambuco (IPA).

RESULTS

In the municipalities that comprise the Sertão do Moxotó region, eight species of *Ipomoea* and one species of *Jacquemonthia* (Fig.1) were identified containing variable concentrations of swainsonine and calystegines (Table 1). Most species were found in Sertânia (seven in total). In this municipality the average rainfall in the last three months that preceded the collection of plants was 36.6mm and the annual average rainfall was 34mm, with more significant rains occurring from March to July. The species most observed and in higher amounts was *Ipomoea sericosepala*, which contained only calystegines (B1 0.013%, B2<0.001% and C1 0.002%) and *I. brasiliana*, which also contained only calystegines (B1 0.019%, B2 0.005% and C1 0.031%). Two other species were also observed frequently, however one contained both swainsonine and calystegines in its composition, identified as *I. megapotamica* (swainsonine 0.016%, calystegines B1 0.024%, B2 0.001%, B3 0.002% and C1 0.003%) and the other one, identified as *I. subincana* contained only swainsonine, in the concentration of 0.011%. *Ipomoea sericosepala* and *I. brasiliana* were observed both on the main highways and bordering roads, covering fences of the farms and shrubs in most of rock outcrops. At the collection sites, numerous sprouts were observed, as well as well-developed adult individuals up to 2.5m in height and abundant leaf mass. In the rock outcrops, where usually goats and sheep gather for pasture it was common to observe these animals ingesting three or four species of *Ipomoea* that vegetated simultaneously, some covering the others (Fig.2). In this situation it was possible to observe specially *I. sericosepala*, *I. brasiliana*, *I. megapotamica* and *I. subincana*. The other species of *Ipomoea* were not so abundant and were only seen occasionally; however, they contained concentrations of both swainsonine and calystegines.

In Manari, three species of *Ipomoea* and one of *Jacquemontia* were observed. In this municipality the average rainfall of the last three months previous the collection of plants was 81.3mm and the annual rainfall was 67.5mm, with significant rains occurring from April to August (Fig.3). The main species observed was *I. sericosepala*. The mean concentration of swainsonine in this species was 0.011% and the concentration of calystegines B1, B2, B3 and C1 was 0.012%, 0.001%, 0.002% and 0.003% respectively. *Ipomoea bahiensis* was the second more frequent species observed; the analyses identified only calystegines B2 (0.061%) and C1 (0.002%) in this plant composition. *Jacquemontia corymbulosa* was the third more common species found both on the roads and near the fences of the farms, forming an abundant leaf mass, sometimes covering extensive areas of soil and pasture. The mean concentration of both swainsonine and calystegine B2 in these species was 0.01%. No concentrations of indolizidine alkaloids were detected in *Ipomoea rosea* collected from this region.

Table 1. Eight species of *Ipomoea* and one specie of *Jacquemonthia* identified containing variable concentrations of swainsonine and calystegines from Sertão do Moxotó region, Pernambuco, Brazil

Month of collection	Municipality	Total rainfall[a]	Voucher number	Species	Swainsonine[b]	Calystegines			
						B1	B2	B3	C1
April	Sertânia	36,6mm	60018	*Ipomoea sericosepala*	-[c]	0.013	<0.001	-	0.002
			60019	*Ipomoea brasiliana*	-	0.019	0.005	-	0.031
			60020	*Ipomoea subincana*	0.011	-	-	-	-
			60021	*Ipomoea megapotamica*	0.016	0.024	0.001	0.002	0.003
			60022	*Ipomoea rosea*	0.001	-	-	-	-
			60023	*Ipomoea bahiensis*	-	-	0.001	-	-
			60024	*Ipomoea incarnata*	-	0.003	0.022	0.001	0.006
May	Betânia	250mm	60025	*Ipomoea nil*	-	0.003	0.007	-	0.001
			60026	*Ipomoea sericosepala*	0.012	0.013	0.001	-	0.002
June	Ibimirim	229mm	60027	*Ipomoea rosea*	0.007	0.001	0.003	-	<0.001
June	Manari	81,3mm	60028	*Ipomoea rosea*	-	-	-	-	-
			60029	*Jacquemontia corymbulosa*	0.001	-	0.001	-	-
			60030	*Ipomoea brasiliana*	-	0.029	0.014	-	0.052
			60031	*Ipomoea bahiensis*	-	-	0.061	-	0.002
			60032	*Ipomoea sericosepala*	0.011	0.012	0.001	0.002	0.003
July	Custódia	414mm	60033	*Ipomoea nil*	-	-	-	-	-
July	Inajá	179mm	60034	*Ipomoea brasiliana*	-	-	<0.001	-	0.001
July	Arcoverde	412mm	60035	*Ipomoea nil*	-	0.002	0.008	-	0.001

[a] Total rainfall of the month and the last three months that preceded the collection of plants, [b] concentrations of swainsonine equal to or greater than 0.001% of dry matter are considered toxic to herbivores (Molyneux et al. 1995), [c] species in which the presence of swainsonine has not been detected are symbolized by a dash (-).

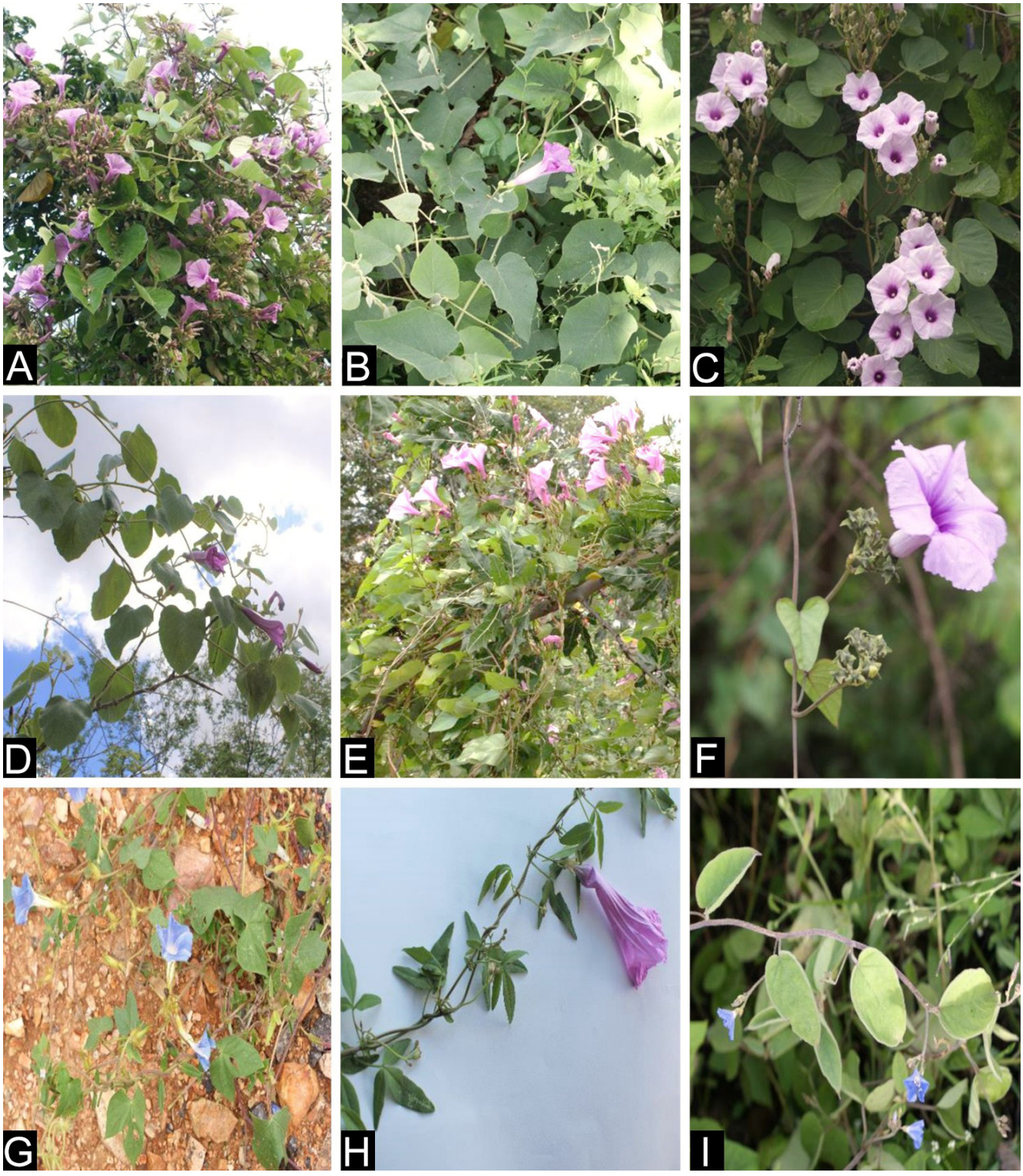

Fig.1. Species of *Ipomoea* in flowering phase during the rainy season of 2017, Sertão do Moxotó/PE, Brazil. (**A**) *Ipomoea sericosepala*, (**B**) *Ipomoea brasiliana*, (**C**) *Ipomoea megapotamica*, (**D**) *Ipomoea subincana* and (**E**) *Ipomoea incarnata* in April, municipality of Sertânia. (**F**) *Ipomoea bahiensis* in May, municipality of Manari. (**G**) *Ipomoea nil* in May, municipality of Betânia. (**H**) *Ipomoea rosea* in June, municipality of Manari. (**I**) *Jacquemontia corymbulosa* in May, municipality of Manari.

In Betânia, the most species observed was *I. sericosepala* and *I. nil* was seen occasionally, occurring only at borders of highways. The average rainfall in the period before the collection was 73.7mm with intense raining indexes from March to April. Swainsonine concentration in *I. sericosepala* was 0.012% and calystegines B1, B2 and C1 were 0.013%, 0.001% and <0.002%, respectively. Only calystegines B1, B2 and C1 were detected in *I. nil*, the average concentrations were

Fig.2. Pasture areas of goats in Sertão do Moxotó/PE, Brazil. (**A**) Pasture mainly formed by *Froelichia humboldtiana* and shrubs of *Ipomoea brasiliana* in sprouting phase (arrows), Inajá, July of 2017. (**B**) Caatinga area in the rainy season, with available forage and despite this, goats present predilection for *Ipomoea sericosepala* and (**C**) goat showing preference for *Ipomoea megapotamica* instead of other forages during the rainy season.

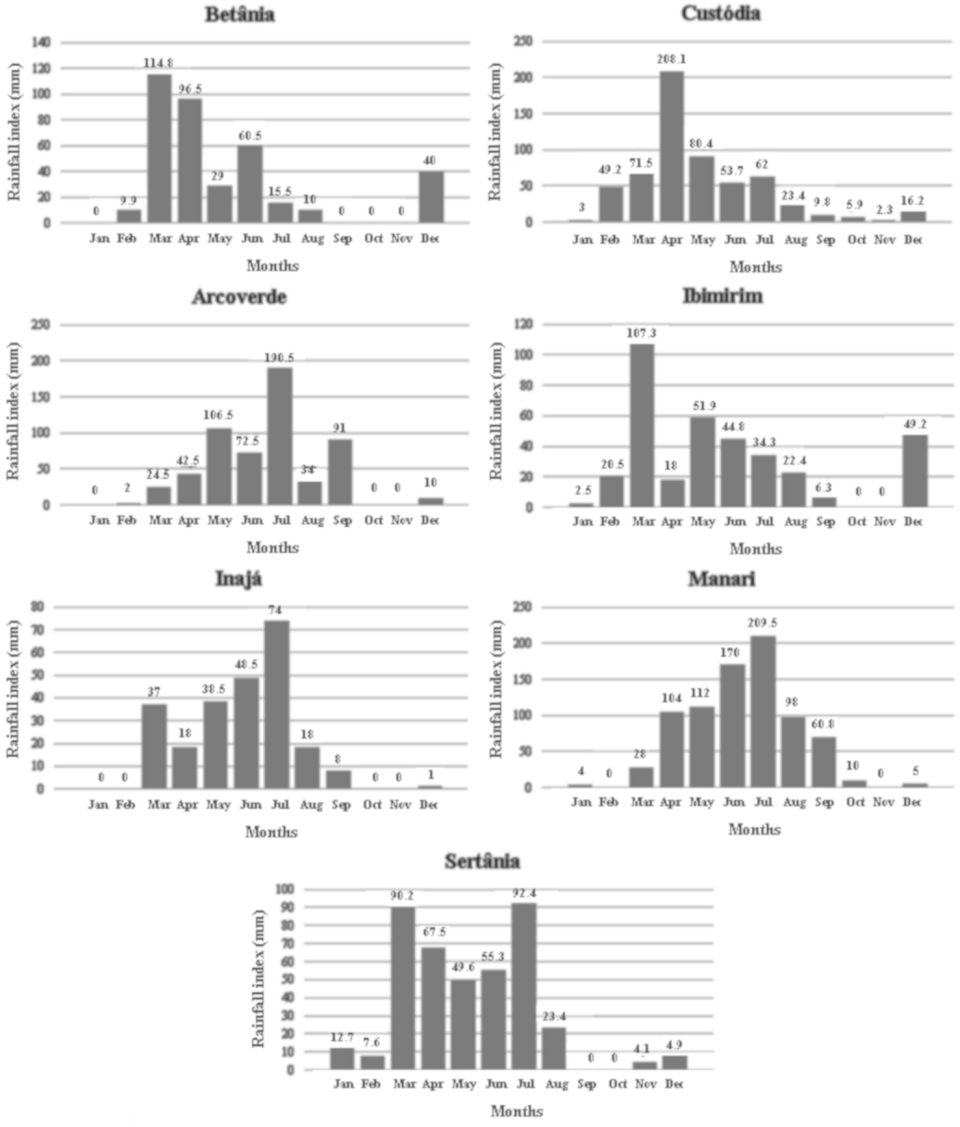

Fig.3. Pluviometric index in the municipalities that comprehend the Sertão do Moxotó region, Brazil. Notice March, April, May, June and July months had higher levels of rainfall, these periods could be associated with presence of Convolvulaceae in the studied region and when cases of lysosomal storage disease may occur.

0.003%, 0.007% and 0.001%, respectively. In Custódia and Ibimirim only two species of *Ipomoea* were observed, one in each municipality. The mean rainfall in Custódia in the period before the collection was 73.8mm with intense raining indexes from May to July; In Ibimirim the mean rainfall was 61.4mm with intense raining period from March to May. In Custódia only the occurrence of *I. nil* was observed and the presence of indolizidine alkaloids was not detected. The mean rainfall in the period before the collection of *I. nill* in Custódia was 117.4mm with rains from April to May. In *I. rosea* collected from Ibimirim, swainsonine concentration was 0.07% and calystegines B1, B2 and C1 were 0.001%, 0.003% and <0.001% respectively. In both municipalities amounts of these plants were not expressive and the observation was occasional.

In Arcoverde only *I. nil* was observed; swainsonine was not detected in this plant and concentrations of calystegines B1, B2 and C1 were 0.002%, 0.008% and 0.001%. In Inajá only *I. brasiliana* was observed, in which contained calystegines B2 (<0.001) and C1 (0.001). In Arcoverde, the average rainfall was 73.8mm with more intense rains from May to July. In Inajá the average was 35mm, with pluviometric indexes with below-average rainfall, compared to other municipalities. The distribution of *Ipomoea* species in the studied municipalities are displaced in Figure 4.

DISCUSSION

In this study six new species of *Ipomoea* (*I. subincana*, *I. megapotamica*, *I. rosea*, *I. bahiensis*, *I. incarnata* and *I. nil*) and one species of *Jacquemontia* (*J. corymbulosa*) are presented, containing toxic concentrations of swainsonine and/or calystegines. All these species are endemic from the Brazilian caatinga biome (Oliveira et al. 2013) and their importance as toxic plants for livestock must be investigated in all the Brazilian semiarid region. Two species most founded were *I. sericosepala* (previously *Turbina cordata)* (Wood et al. 2015) and *I. brasiliana* (previously reported as *I. marcellia* and *I.* aff. *verbascoidea*) (Mendonça et al. 2012, Rocha et al. 2016, Simão-Bianchini & Ferreira 2015). *Ipomoea sericosepala* has been reported as toxic to goats, cattle and horses (Dantas et al. 2007, Assis et al. 2010, Oliveira Júnior et al. 2013) and *I. brasiliana* so far has only been reported as important to goats (Mendonça et al. 2012, Lima et al. 2013). In previous studies, in both *I. sericosepala* and *I. brasiliana* significant amounts of swainsonine and calystegines were detected. Nonetheless, several samples of both plants were negative for swainsonine. This occurs because the swainsonine concentration varies considerably among species, since the production of this toxin is dependent on the presence of endophytic fungi. Calystegines, on the other hand, are secondary metabolites produced by plants (Cook et al. 2013).

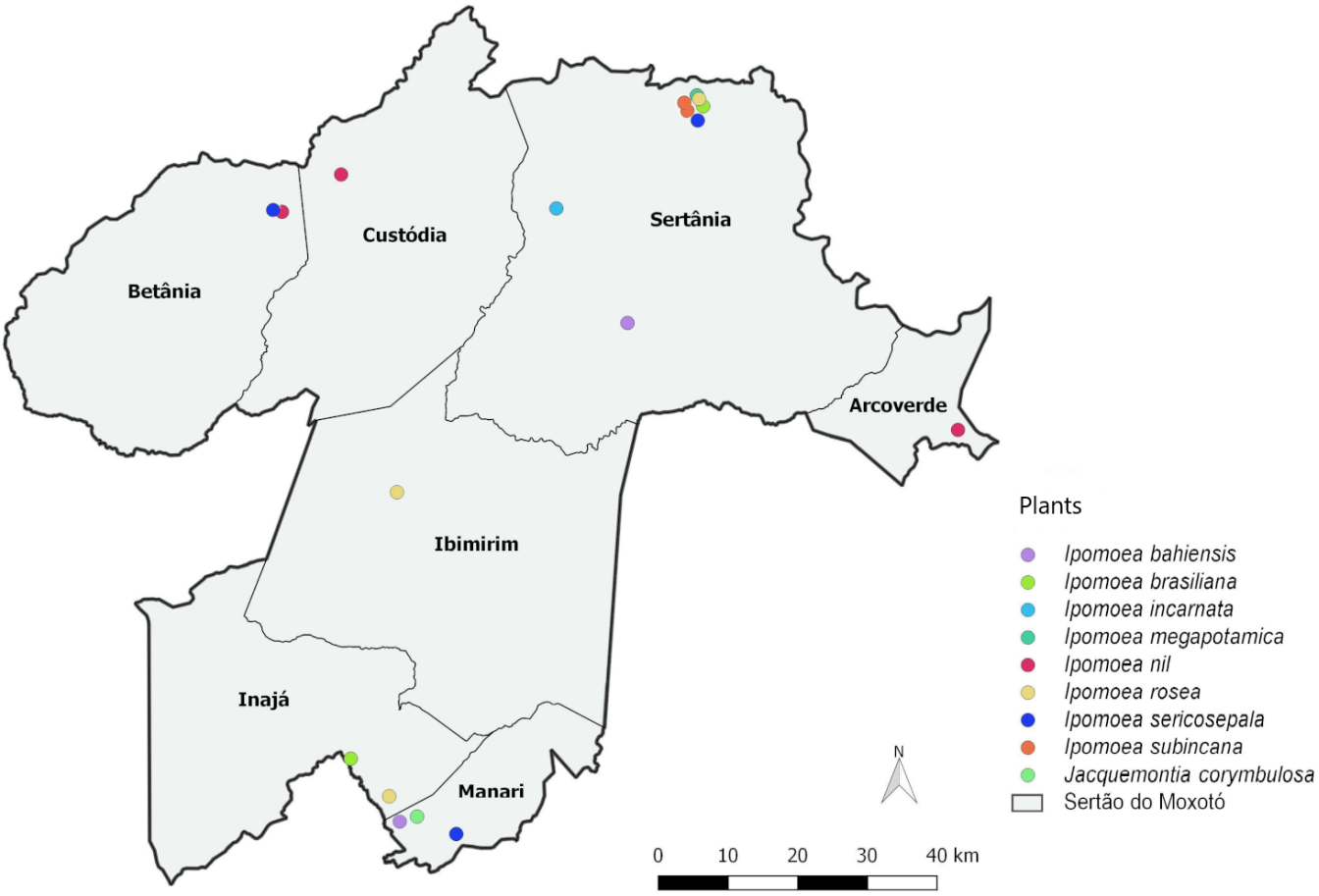

Fig.4. Distribution of the *Ipomoea* species found during the study in Sertão do Moxotó/PE, Brazil. It is observed that some regions have larger variability and predominance of species.

It is important to emphasize these species presented here grows during rainy season and its leaves dry and fall at the beginning of the drought, after the fructification, resprouting again at the beginning of the the rains (Barbosa et al. 2006, Mendonça et al. 2011, 2012). For this reason, it is necessary the study of pluviometric indexes, because high raining's could indicate the most favorable months for poisoning by these plants in ruminants and horses. Thus, considering the average rainfall of 2017 in the Sertão do Moxotó region, the most favorable months to occur outbreaks of lysosomal storage disease in herbivores are from March to July (Fig.3).

Several species of the Convolvulaceae family are non-toxic and are important fodder that should be used as animal feed (Cook et al. 2013), especially in northeastern semiarid region due to the animal food scarcity that frequently occurs in this region. However, several species are toxic to ruminants, horses and wildlife (Cook et al. 2013) and should therefore be studied. Thus, according to the results presented in this study, *I. subincana, I. megapotamica, I. rosea* and *Jacquemontia corymbulosa* presented a significative toxicity potential to herbivores, since they had swainsonine concentrations equal or higher than 0.001% (Molyneux et al. 1995). So far, the toxicity determination of these species still must be proven experimentally. For species containing only calystegines, such as *I. bahiensis, I. incarnata* and *I. nil*, additional studies should be conducted to prove the toxicity of these compounds in ruminants (without simultaneous action of swainsonine). *In vitro*, calystegines are potent inhibitors of hydrolases, but studies in rats have shown negative results on the development of lysosomal storage disease in this species. Another aspect to be considered is that *I. bahiensis, I. incarnata* and *I. nil* may present swainsonine in their composition, similarly to what was observed with *I. sericosepala* and *I. brasiliana* which sometimes presented or not swainsonine concentrations.

Considering our field observations over the past 10 years in the Sertão do Moxotó region about plant poisoning in ruminants, it is important to be noted that, unlike what occurs with *I. carnea* subsp. *fistulosa*, most of the species presented in this study have good palatability and be one of the feeding choices for goats, even when other forages are present. Perhaps because of that, in addition to the wide variety of species that contain swainsonine and calystegines, poisoning by plants containing these alkaloids are so frequent in the northeastern semiarid region, if considered the reported case numbers. Another factor that contributes to the occurrence of poisonings is the social facilitation mechanism, in which animals that start the ingestion of these plants develop the habit of compulsively ingesting them and, by influence, induce other animals of the same species to ingest them (Tokarnia et al. 1960, Driemeier et al. 2000, Colodel et al. 2002, Dantas et al. 2007, Barbosa et al. 2007, Oliveira et al. 2009, Mendonça et al. 2012).

CONCLUSIONS

The discovery of new species of *Ipomoea* and *Jacquemontia* containing toxic polyhydroxy alkaloids reinforces the importance of this group of poisonous plants for ruminants and horses in the Pernambuco semiarid region.

Epidemiological research must be conducted to investigate the occurrence of lysosomal storage disease associated with these new species.

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