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Adverse Effect of Cashew Nuts Studied on Ants as Biological Models

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ABSTRACT

Cashew nuts have several medicinal beneficial properties and contain many elements beneficial for our health. However, they produce kinds of substance, the urushiol, which can cause severe dermatitis by contact or by ingestion. In the present work, we studied ethological and physiological effects of these nuts, using ants as biological models. Effectively, these nuts are good for the health though they induce stress, excitation, and nervousness. Moreover, we also discovered a novel not yet revealed effect of these nuts which must imperatively be known by consumers. After weaning, the effects of cashew nut rapidly decreased with two quick decreases, one from 0 to 3 hours and anther one from 18 to about 20 hours. These two decreases can be perceived by consumers who are then tempted to ingest again cashew nuts. Such a behavior constitutes a dependence which leads to more and more nuts ingested, what could accentuate the probability of suffering from dermatitis. The solution is limiting the amount of ingested cashew nuts and consumed them together with other kind of nuts or similar fruits not containing urushiol.

Keywords: Addiction, Ant, Dependence, Nervousness, Stress, Urushiol.

ABBREVIATIONS

ang.deg. = Angular Degrees; ang.deg./cm = Angular Degrees per Centimeter; mm/s = Millimeter per Second; χ^2= Chi-Square; vs = Versus; n° = Number; cm = Centimeter; mm = Millimeter; ml = Milliliter; mg = Milligram; s = Second; min = Minute; h = Hour; t = Time; % = Percentage.

INTRODUCTION

Cashew nuts have been shown to have several nutritional qualities. They contain polyunsaturated fatty acid which are essential for the cellular membrane and which can prevent from cardio vascular diseases. They also contain many vitamins such as vitamin K (essential for calcium fixation and blood coagulation), vitamin B9 (folic acid), vitamin B6 as well as B1. Compared with other kinds of nuts, cashew nuts are very rich in protein. Moreover, they contain a large amount of iron, as well as of several mineral ions such as calcium and potassium. All these properties are effective in the living organisms who feel better, in better health. These informations can be found in several internet sites such as: https://fr.wikipedia.org>wiki>Noix_de_cajou; https://family-crops.

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squaresite>product>noixdecajou; https://www.amazon.fr>cashew-nuts>k=cashew+nuts [1-3].

Unfortunately, as reported in several publications such as those of Baer, Acadpharm and Symes & Dawson [4-6], cashew nuts contain a small amount of a substance, named urushiol, potentially dangerous, and the nutritionist advices consist in consuming a mixture of several kinds of nuts, for instance, cashew nuts together with Brasile nuts, Pecan nuts, nuts of walnut, hazelnut. Out of cashew nuts, urushiol produces a kind of warmish and a small amount of a volatile substance which may cause severe dermatitis. Also, cashew nuts cause several health problems such as digestive ones, tiredness, headache (see the Discussion section). Already in 1993, the properties of cashew nuts have been examined by Oloda & Carke [7]. More recently, the scientific and technological monitoring of this fruit has been reviewed by Olivera et al. [8]. The culture and the supply of cashew nuts are essentially performed in Nigeria [9].

In the same way the physiological and etiological effects of products used by humans have been studied (67 products have already been studies), the potential and negative effects of cashew nuts consumption was examined in the present work, using ants as biological models. Doing so revealed an unexpected not yet known effect of cashew nuts, valuable for humans, which must imperatively be related to consumers of this kind of fruit. This experimental work is here related after having explained why using ants as models, which species was used and what is known about it, and which traits were examined.

Biological processes are the same in most animal species (f.i., [10], genetics, nervous influx, muscles contraction, sensory perception, conditioning acquisition). Consequently, they are generally firstly studied on vertebrates or invertebrates, then in humans [11]. Invertebrates are often preferred because they are small, can be maintained in a laboratory at low cost, and present a short reproductive cycle [12]. Hymenoptera (e.g., bees) are often used [13], and ants can thus be used. They can be the more so because several colonies containing hundreds of individuals can be maintained during long time times, at low cost, and very easily. We are accustomed to work on the species *Myrmica sabuleti* Meirnert, 1861. We know rather well its biology. Among others, we have examined their visual perception, navigation, recruitment [14], ontogenesis of some of their skills [15], self-recognition in a mirror [16], distance and size effects [17], Weber's law [18], numerosity abilities and related topics [19-22]. With so many known skills, this species constitutes a comfortable biological model. The ethological and physiological traits we intended to examine were the food intake, locomotion, audacity, tactile perception, social relationships, stress, cognition, learning and memory, adaptation to side effects, dependence on consumption, and decrease of the effect after weaning.

MATERIALS AND METHODS

Note

The materials and methods used to make the present work were very similar to those used for previous works (for instance [23-25]). Having been detailed in many previous publications (about 67 ones), as well as summarized in until now eight mini reviews, they are here only briefly related, but still reported for the readers' convenience. Of course, doing so inevitably leaded to self-plagiarism.

Collection and Maintenance of ants

The experiments were made on two large colonies collected in June 2022, in Ardennes (Belgium), from an abandoned quarry located in the Aise valley. These colonies lived under stones and in grass. They contained about 500 to 1,000 workers, a few queens and brood. In the laboratory, they were maintained in 1 to 3 glass tubes half-filled of water, with a cotton plug for separating the water and the part devoted to the ants. The nest tubes of each two colonies were set in a tray the borders of which having been slightly talked. The trays served as foraging area; food was there delivered. The food consisted in larvae of *Tenebrio molitor* (given three times per week) and in sugar water (permanently delivered in small plugged tubes). The lighting of the laboratory equaled 330 during the day and 110 lux during the night; the humidity always equaled 80% and the temperature constantly 20°.

Solution of cashew nuts given to the ants

A package of 73 cashew nuts weighing 107 gr was bought. Each nut had thus a weight of 17, 056 gr. Normally, humans consume about 25 gr of these nuts per day, so together with one liter of water, thus 25 gr of nuts 'into' 1,000 ml water. Due to their physiology (excretory apparatus) and anatomy (cuticle), the insects, and thus ants, consume about ten less water than mammals. Consequently, to maintain ants under a diet with cashew nuts similar to that of humans consuming this product, they must receive a solution of 25 gr of cashew nuts into 100 ml of water, or 10,2 gr into 60 ml of water, or in fact into 60 ml of their usual sugar water. We thus realized the latter solution (see Figure 1), pulverized it and gave it to the ants in their usual cotton-plugged tubes. The plug of these tubes was refreshed every 2-3 days; their content was

renewed every seven days. Several times per day, we checked if ants drunk the given solution, and they did. We firstly made the control experiments on the two used colonies maintained under normal diet. Then, we replaced the ants' tubes filled of sugar water by tubes containing the nuts, and we started performing the test experiments 12 hours later.

Figure 1. Realization of the solution of cashew nuts given to the ants. Successively: upper part: the bought pack of these nuts, the information given on it, the number of nuts used for making the solution given to the ants; lower part: the crush of the nuts, an ant drinking the solution of nuts, a nest used for the experiments.

Meat and sugar water consumption, general activity

For ants under normal diet, then for those having cashew nuts in their sugar water, we counted four times per day during six days (total number of counts = 4 x 2 x 6 = 48 counts), the ants staying on the meat food, those present at the entrance of the sugar water tube, and those being active at any place of their environment (foraging area, nest entrance, inside the nest ...etc....). For each kind of count and each diet, we established the daily mean (Table 1, lines 1 - 6), and the six daily means obtained for ants having cashew nuts in their sugar water were compared to those obtained for ants under normal diet using the non-parametric test of Wilcoxon [26]. In addition, for each kind of diet and of count, we established the mean of the six daily means (Table 1, line I-VI).

Linear and angular speeds; orientation to a tied nestmate

These three traits were quantified on ants moving in their foraging area, the linear and the angular speeds without stimulating the ants; the orientation while stimulating them with a nestmate tied to a piece of paper (Figure 2A).

A tied nestmate emits its attractive mandible glands alarm pheromone what attracted the ants moving all around. For the ants' linear and angular speeds as well as for the ants' orientation, 40 trajectories were recorded then analyzed using appropriate software [27]. The software was elaborated on the following definitions. The linear speed (in millimeter per second = mm/s) is the length of a trajectory divided by the time spent to travel it; the angular speed (in angular degrees per centimeter = ang.deg./cm) is the sum of the angles made by successive adjacent segments, divided by the length of the trajectory; the orientation (in angular degrees = ang. deg.) to a location is the sum of successive angles made by the direction of the trajectory and the direction towards the location, divided by the number of measured angles. An orientation value lower than 90° means that the observed animal tends to approach the location. An orientation value higher than 90° means that the observed animal tends to avoid the location. For the three used variables, the median and quartiles of the 40 values were established (Table 2, lines 1, 2, 3). Also, for each variable, the distribution of 40 values obtained for ants eating cashew nuts were compared to those obtained for ants living under normal condition using the non-parametric χ^2 test [26].

Audacity

Such a trait was evaluated through the ants' tendency to come onto an apparatus deposited on their foraging area. This apparatus was a cylinder (height = 4 cm; diameter = 1.5 cm) vertically tied to a squared platform (9 cm2), each one build in Steinbach® white paper (Figure 2B). The ants seen on this apparatus were counted 20 times in the course of 10 minutes (n =20 x 2 = 40). We established the mean and the extremes of these counts (Table 2, line 4). The numbers obtained for the two colonies were correspondingly added. Then, the twenty sums were chronologically subdivided two by two into ten numbers, and the ten numbers obtained for ants eating cashew nuts were compared to the ten numbers obtained for ants normally maintained using the non-parametric Wilcoxon test [26].

Tactile (pain) perception

This physiological trait was estimated through the ants' moving on a rough substrate. Indeed, if well perceiving the rough character of such a substrate, the ants walk slowly, sinuously, with difficulty, often touching it with their antennae (Figure 2C). If poorly perceiving such a character, the ants walk not very sinuously, rather rapidly, and they seldom touch the substrate with their antennae. For each two colonies, a piece (3 cm x 2 + 7 + 2 = 11 cm) of n° 280 emery paper was duly folded and inserted into a tray (15 cm x 7 cm x 4.5 cm), dividing so this tray in 3 zone, a first 3 cm long zone, a second 3 cm long one containing the emery paper, and a last one 9 cm long zone. An experiment consisted in setting 25 ants in the first zone of the apparatus, and in assessing their linear and angular speeds when these ants walked on the rough substrate. The 40 values of linear and of angular speeds obtained for ants consuming cashew nuts, as well as those obtained for ants under normal diet were compared to the 40 values previously obtained on a normal substrate (see an above paragraph) by using the non-parametric χ^2 test [26]. These two comparisons furnished a valuable evaluation of the impact of the cashew nuts on the ants' tactile perception. In addition, for each kind of diet, the 40 recorded values of linear and of angular speeds of ants moving on the rough substrate were characterized by their median and quartiles (Table 2, lines 5, 6).

Brood caring behavior

For each two colonies, a few larvae were removed from the nest and were deposited in front of its entrance. Five of them were observed during five minutes. In fact, the ants' behavior towards them was observed (Figure 2D). The numbers of not re-entered larvae were counted 30 seconds, 1, 2, 3, 4, and 5 minutes after their deposit (Table 3, line 1). We observed only five larvae per colony because we must do so simultaneously. We did not repeat the experiment because removing brood from the nest largely perturbates the colony. The six numbers of not re-entered larvae obtained for the two colonies were correspondingly added, and the six sums obtained for ants eating cashew nuts were compared to the six sums obtained for ants normally maintained using the non-parametric test of Wilcoxon [26].

Social relationships

Social animals belonging to the same group or colony do not aggress themselves. Ants are such animals. However, ingested food or environmental parameter may affect this peaceful behavior. To know if cashew nuts present such an impact, for each two colonies, under each kind of diet, five dyadic encounters between nestmates were performed. These encounters were conducted in a talked cup (diameter = 2cm, height = 1.6cm). Each time, one ant of the pair was carefully observed during 5 minutes. Its behavior was assessed by the numbers of times it did nothing (level 0 of aggressiveness), touched the other ant with its antennae (level 1), opened its mandibles (level 2), gripped and/or pulled the other ant (level 3), and tried to sting or stung the other ant (level 4) (Table 3, line 2; Figure 2E). For each of

these five levels of aggressiveness, the numbers obtained for the 10 observed ants were correspondingly added. Doing so allowed obtaining a distribution of values for each kind of diet, and the distribution corresponding to ants consuming cashew nuts were compared to that corresponding to ants normally maintained using the non-parametric χ^2 test [26]. In addition, for each kind of diet, a variable 'a' was calculated, 'a' being equaled to the number of aggressiveness levels 2 + 3 + 4 divided by the number of aggressive levels 0 + 1 (Table 3, line 2).

State of stress and cognition

To be able to escape from an enclosure, an individual must stay calm, not stress, look for an exit, as well as having intact its cognitive ability. To estimate the impact of cashew nuts on the ants' state of stress and cognition, for ants normally maintained and for those consuming the nuts, for each two colonies, six ones were enclosed under a reversed talked cup (made of polyacetate; height = 8cm, bottom diameter = 7 cm, ceiling diameter = 5 cm) deposited in their foraging area. A notch (3 mm height, 2 mm width) has been performed in the rim of the bottom of this cup in order to give to the ants the possibility to escape (Figure 2F). For each colony, the numbers of escaped ants among the six enclosed ones were counted after 2, 4, 6, 8, 10 and 12 minutes. The numbers obtained for the two colonies were correspondingly added (Table 3, line 3). The six sums (those for 2, 4, 6, 8, 10 and 12 min) obtained for ants consuming cashew nuts were compared to the six ones obtained for ants normally maintained using the non-parametric Wilcoxon test [26].

Cognition

This physiological trait was assessed thanks to the ants' ability to cross a twists and turns path. For each colony, two folded pieces of Steinbach ® paper (4.5 cm x 12 cm) were inserted in a tray (15 cm x 7 cm x 4.5 cm) dividing the tray into 3 zone, a 2 cm long zone, a zone containing the twists and turns path, and 8 cm long zone beyond this 'difficult' path (Figure 3A). To conduct an experiment on a colony, 15 ants were set into the first zone located in front of the twists and turns path, and the ants still in this zone as well as those having reached the third zone lying beyond this path were counted after 2, 4, 6, 8, 10 and 12 minutes. The numbers obtained for the two colonies were correspondingly added (Table 3, line 4), and for each of the two considered zone, the six added numbers (i.e., for 2, 4, 6, 8, 10, 12 min) obtained for ants consuming the nuts were compared to those obtained for ants normally maintained using the non-parametric Wilcoxon test [26].

Visual operant conditioning and memory

At a recorded time, a blue hollow cube made in Canson® paper was deposited above the entrance of the tube containing the sugar water and the meat was set near this tube (Figure 3B 2a). The ants were so submitted to operant visual conditioning. The experiment on ants living under normal diet had previously been done on another similar colony of *M. sabuleti* collected in the same site at the same time, because when an individual has acquired conditioning to a stimulus, it keeps this conditioning during a rather long time, and even after having forgotten it, it more rapidly than usually acquires it again. It can thus no longer be used to assess its conditioning acquisition. Over the ants' conditioning acquisition, and after the blue cube removal over their loss of conditioning, the ants were tested four times in a talked Y-maze made of strong white paper, deposited in a tray, and into which a blue hollow cube had been randomly set in one of its branches. To conduct a test on a colony, 10 ants were one by one transferred into the maze before its division into two branches, and the ants' choice of one branch was recorded (Figure 3B 2b). Choosing the branch containing the blue cube was considered as giving the correct response. After having been tested, the ant was kept in a talked cup to avoid testing twice the same ant. When the 10 ants of a colony were tested, all of them were transferred again into their foraging area. For each test, the ants' responses obtained for the two colonies were added, and the proportions of correct responses each time established (Table 4). The proportions obtained for ants consuming cashew nuts were compared to those obtained for ants normally maintained using the non-parametric Wilcoxon test [26].

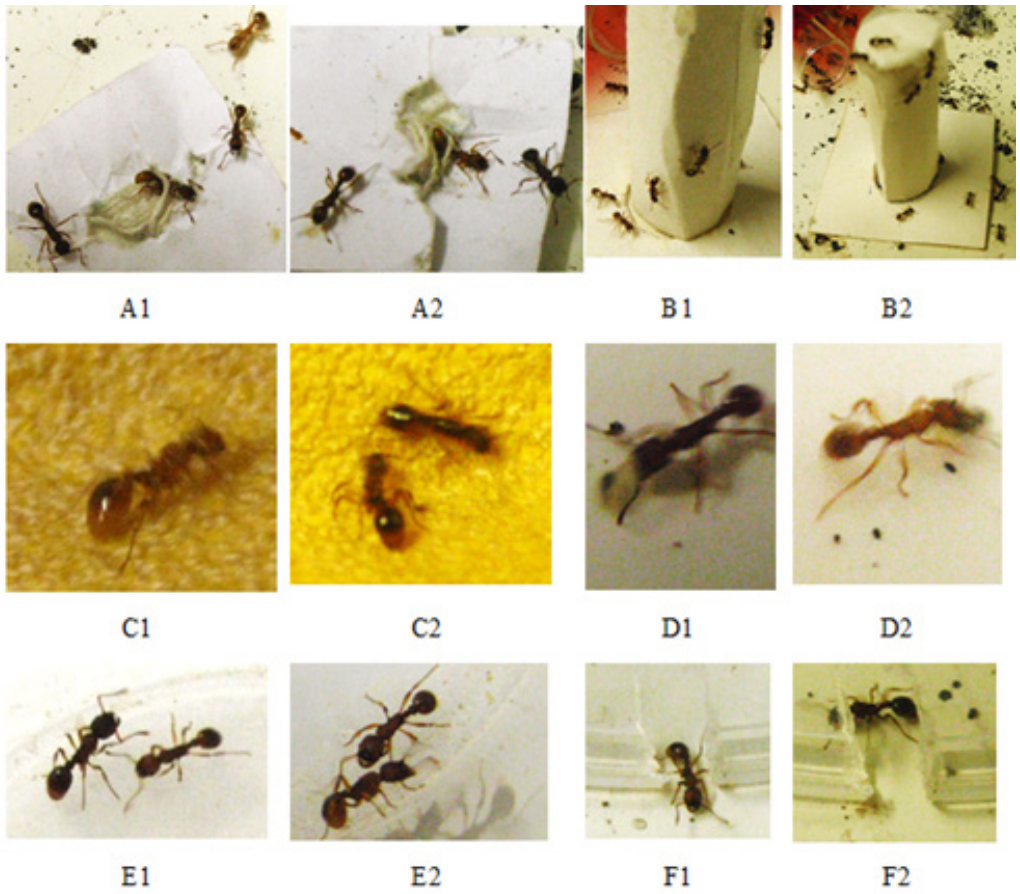


Figure 2. Effects of cashew nuts on eight physiological or ethological traits. 1: ants under normal diet; 2: ants under a cashew nuts diet. 1: A: ants coming onto a tied nestmate very well under normal diet, but not so well while consuming cashew nuts; B: ants coming onto an unknown apparatus, doing so obviously more while consuming cashew nuts (going higher on the tower); C: ants walking with difficulty on a rough substrate whatever their diet; D: ants taking care of a larva whatever their diet; E: nestmates staying near each other without aggressiveness, essentially while living under normal diet; F: an ant under normal diet going out of an enclosure, but not doing so while consuming cashew nuts.

Adaptation to side effects

Adaptation to a product occurs when a consumer less and less suffers over time from the adverse effects of this product. To study such an adaptation, an adverse effect of the product must be quantified soon after the individual consumed the product, and then later, after it consumed it for a longer time; and the two assessments must be compared. In this work, the ants' angular speed was affected by cashew nuts. So, this trait assessed after one day of consumption was again assessed after eight days of consumption, and the obtained distributions of the 40 variables were compared to one another using the non-parametric χ^2 test [26]. Moreover, the median and quartiles of the values recorded after 8 days of consumption were established (Table 5, upper part).

Dependence

Dependence on a product occurs when a consumer wants to continuously have the product at his disposal, consumes it whatever its adverse effects, and finally can no longer live without consuming it. In this work, the ants' dependence on cashew nuts was examined after the ants had this fruit during 10 days. For each colony, 15 ants were transferred into an own talked tray (15cm ×7 cm × 5cm) inside of which two cotton-plugged tubes (length = 2.5 cm, diam. = 0.5 cm) had been deposited, one containing sugar water, the other contained the sugared solution of cashew nuts used all over the experimental work. The tube containing the nuts was set on the right of the tray of one colony and on the left of the tray of the other one (Figure 3 C2). Then, the ants sighted

at the entrance of each tube were counted 15 times over 15 minutes, and for each colony and each kind of count, the recorded numbers were added. The two obtained sums for each two colonies were correspondingly added, and the resulting sums allowed calculating the proportion of ants seen in front of each kind of tube (Table 5, lower part). Also, the two resulting sums were compared to the two numbers expected if the ants randomly visited each kind of tubes using the non-parametric χ^2 goodness-of-fit test [26].

Decrease of the effects after the end of consumption

The decrease of the effect of cashew nuts was examined after the ants consumed these nuts during 12 days. Twelve hours before this study, the ants received a fresh sugared solution of cashew nuts, and the ants' angular speed was assessed as it has been assessed after one as well as eight days of consumption, except that 20 instead of 40 ants' trajectories were recorded and analyzed. This reduction was used in order to be able to assess the successive angular speed obtained all along the decrease of the effect of cashew nuts, in other words, to evaluate the current situation. After this assessment, made at a time we named t = 0, the ants' tubes containing the nuts solution were replaced by tubes containing sugar water, and this change constituted the start of the weaning. Since that 'weaning' time, the ants' angular speed was assessed every three hours until the obtained value became similar to the control one (to that obtained for ants under normal maintenance). For each assessment, the median and quartiles of the 20 recorded values were established (Table 6), and were graphically presented (Figure 4). The successively obtained distributions of angular speed values were compared to the distribution obtained at t = 0 as well as to the control distribution using the non-parametric χ^2 test for independent samples (Table 6) [26]. In addition, the mathematical functions best describing the observed decrease of the effect of cashew nuts was given in the text.

RESULTS

Food intake, general activity

These physiological traits were impacted by cashew nuts consumption (Table 1). Indeed, under that fruit diet, the ants consumed less meat and less sugar water, and were less active than while living under normal diet. These three decreases were statistically significant (N = 5, T = 15, P = 0.031). However, in the course of time (after three to four days), their food consumption and their activity increased. The ants may thus adapt themselves to the adverse effect of cashew nuts, a presumption we experimentally checked (see the paragraph on the ants' adaptation). After several days, the ants were numerous on the sugar water. This may result from some dependence on cashew nuts consumption, a presumption examined in a following experiment (see the paragraph concerning the ants' dependence).

Table 1. Impact of cashew nuts on food intake and general activity. The table gives the mean numbers of ants sighted each day, during six days, on their meat and on their sugar water, as well as of those being active at any place (line I to VI), and the mean of these six daily means (line I-VI). The nuts impacted these three traits, but sugar water consumption then increased over time.

Days	Under normal diet	Under a diet with cashew nuts
	meat sugar water activity	meat sugar water activity
I	2.50 5.75 24.00	1.37 2.75 13.50
II	2.38 5.00 18.75	1.00 2.50 17.25
III	2.25 5.00 24.25	1.50 2.25 18.75
IV	2.63 4.63 22.25	1.50 3.75 15.00
V	3.00 4.88 22.13	1.63 3.50 14.12
VI	2.75 4.75 21.50	1.75 3.50 12.50
I-VI	2.59 5.00 22.15	1.45 3.08 15.19

Table 2. Impact of cashew nuts on five ethological and physiological traits. The table gives the median (and quartiles) or the mean [and extreme] of the obtained variable values. The nuts impacted the ants' angular speed, orientation, and audacity.

Traits	Under normal diet	Under a diet with cashew nuts
Linear speed (mm/s)	7.1 (6.7 – 8.5)	7.9 (6.8 – 8.7)
Angular speed (ang.deg./cm)	99 (87 – 118)	164 (152 – 188)
Orientation (ang.deg.)	28.2 (21.9 – 39.4)	58.8 (46.3 – 76.6)
Audacity (n° of ants)	3.23 [2 – 5]	6.57 [4 –9]
Tactile perception		
Linear speed	3.0 (2.7 – 3.4)	3.7 (3.1 – 4.1)
Angular speed	307 (267 – 343)	299 (248 – 337)

Linear and angular speed

While consuming cashew nuts, the ants walked more sinuously than while living under normal diet, this difference being statistically significant ($\chi = 45.45$, df = 1, $P < 0.001$) (Table 2, line 2). However, they waked at a similar linear speed (Table 2, line 1) ($\chi = 1.90$, df = 1, $0.10 < P < 0.20$). As deduced from the first experiment, the ants consuming cashew nuts appeared to be nervous, excited, presenting erratic displacement, with frequent turning and quick changes of movement. Following experiment tried to check this observation.

Orientation

The ants consuming cashew nuts oriented themselves less well towards a tied nestmates than ants living under normal diet (Table 2, line 3; Figure 2A), a difference statistically significant (Figure 2A) ($\chi = 26.59$, df = 1, $P < 0.001$). This may result from their erratic displacement, but could also be due to a less perception of the attractive pheromone emitted by the tied nestmate. A following experiment thus checked the intact value of the sensory perception of the ants consuming cashew nuts.

Audacity

The ants consuming cashew nuts were far more audacious than those living under normal diet: more ants came onto the experimental apparatus and climbed onto the tower (Figure 2B; Table 2, line 4) (N = 10, T = -55, P = 0.001). This was in agreement with the results relative to the angular speed and the orientation: the ants consuming cashew nuts were excited, nervous.

Tactile perception

The ants consuming cashew nuts had the same tactile perception than those living under normal diet (Table 2, lines 5, 6; Figure 2C). Indeed, on a rough substrate, under the tow two kinds of diet, their linear speed was lower and their angular speed higher than those presented on a normal substrate. Statistically, we obtained for normal diet: linear speed: $\chi = 76.09$, df = 1, $P < 0.001$, angular speed: $\chi = 72.37$, df = 1, $P < 0.001$; and for diet under cashew nuts: linear speed: $\chi = 48.00$, df = 1, $P < 0.001$, angular speed: $\chi = 65.45$, df = 1, $P < 0.001$.

Brood caring

This ethological trait was not affected by cashew nuts consumption (Table 3, line 1; Figure 2D). While consuming this fruit, the ants took care of their brood exactly as while living under normal diet (N = 2, NS). Thus, their nervousness did not impact their brood caring behavior.

Table 3. Impact of cashew nuts on four ethological and physiological traits. The table gives the numbers of ants or behavior observed over time or during 5 minutes. The nuts affect the ants' escaping ability, and thus their stress, nervousness, excitation.

Trait	Normal diet	Diet with cashew nuts
N° of not re-entered larvae over 5 min	30'' 1' 2' 3' 4' 5' 10 8 4 4 0 0	30'' 1' 2' 3' 4' 5' 10 6 4 2 0 0
N° of presented levels of aggressiveness; variable 'a'	0 1 2 3 4 'a' 52 41 7 0 0 0.06	0 1 2 3 4 'a' 42 42 23 0 0 0.28
N° of escaped ants over 12 min	2' 4' 6' 8' 10' 12' 3 5 9 10 11 12	2' 4' 6' 8' 10' 12' 0. 0 0 0 2 3
N° of ants in front (f) and beyond (b) a twists and turns path over 12 min	2' 4' 6' 8' 10' 12' f: 18 14 10 6 8 4 b: 0 0 2 6 7 7	2' 4' 6' 8' 10' 12' f: 20 17 25 13 10 9 b: 0 1 4 4 7 8

Social relationships

This social behavior was somewhat affected by cashew nuts consumption (Table 3, line 2; Figure 2E), but this was obviously due to the nervousness of the reacting ants: they seldom stayed side by side, doing nothing, and rather often opening their mandibles, a result slightly significant (χ = 9.81, df = 2, P ≤ 0.01).

Escape behavior

This ethological trait was affected by cashew nuts consumption due to the stress, nervousness and excitation of the reacting ants (Table 3, line 3; Figure 2F). Indeed, while consuming this fruit, the ants moved erratically, poorly perceiving the exit of the enclosure, and so, not often going out of this enclosure. This result was statistically significant: N = 6, T = -21, P = 0.016.

Cognition

This trait was not highly affected by cashew nuts consumption (Table 3, line 4; Figure 3A). The stressing ants moved erratically and delayed to leave the area lying in front of the twists and turns path, a result statistically significant (N = 6, T = +21, P = 0.016). However, they then correctly and rapidly crossed the difficult path and reached the area lying beyond it. This time, the difference between the ants under the two kinds of diet was not significant: N = 4, T = -4.5, +4.5, P = 0.563).

Table 4. Impact of cashew nuts on conditioning acquisition and memory. The nuts did not affect these two traits.

Elapsed time (hours)	Normal diet (control): Conditioning scores (%)	+ cashew nuts: n° of correct *vs* wrong responses of colonies A and B A, B; overall scores (%)
24h	75%	7 *vs* 2, 8 *vs* 2 ; 75%
48h	85%	9 *vs* 1, 8 *vs* 1 ; 85%
Cue removal		
24h	80%	9 *vs* 1, 8 *vs* 1 ; 85%
48h	85%	8 *vs* 2, 8 *vs* 2 ; 80%

Conditioning acquisition, memory

This skill was not affected by cashew nuts consumption (Table 4; Figure 3B). Indeed, ants consuming this fruit acquired conditioning and kept its memory exactly as those living under normal diet. This result was in agreement with those obtained for ants crossing a twists and turns path (see the previous experiment).

Table 5. Upper part: adaptation to the side effect of cashew nuts on the ants' angular (ang.deg./cm) speeds: the ants did not adapt themselves to this side effect. Lower part: dependence on cashew nuts consumption: the ants developed significant dependence on this nut consumption.

Measured trait	Normal diet	+ cashew nuts for 1 day	+ cashew nuts for 7 days
Angular speed	99 (87 – 118)	164 (152 – 188)	198 (169 – 226)

Sugared solution	Number and proportion of ants' visits to each solution			
	colony A	colony B	total number	proportion
+ cashew nuts:	42	42	84	65.6%
without these nuts:	12	12	24	34.4%

Adaptation to the side effects of cashew nuts

The result of these experiments, presented in Table 5, upper part, showed that ants did not adapt themselves to the impact of cashew nuts of their sinuosity of movement, so on the nervousness and excitation induced by that nut. Indeed, after eight days of consumption, their sinuosity was even slightly higher than that presented after one day of consumption (198 vs 164 ang. deg./cm; $\chi = 6.06$, df = 1, $0.01 < P < 0.02$). Such a result was again obtained after 12 days of cashew nuts consumption (see the subsection relative to the decrease of the effects of this nut after weaning).

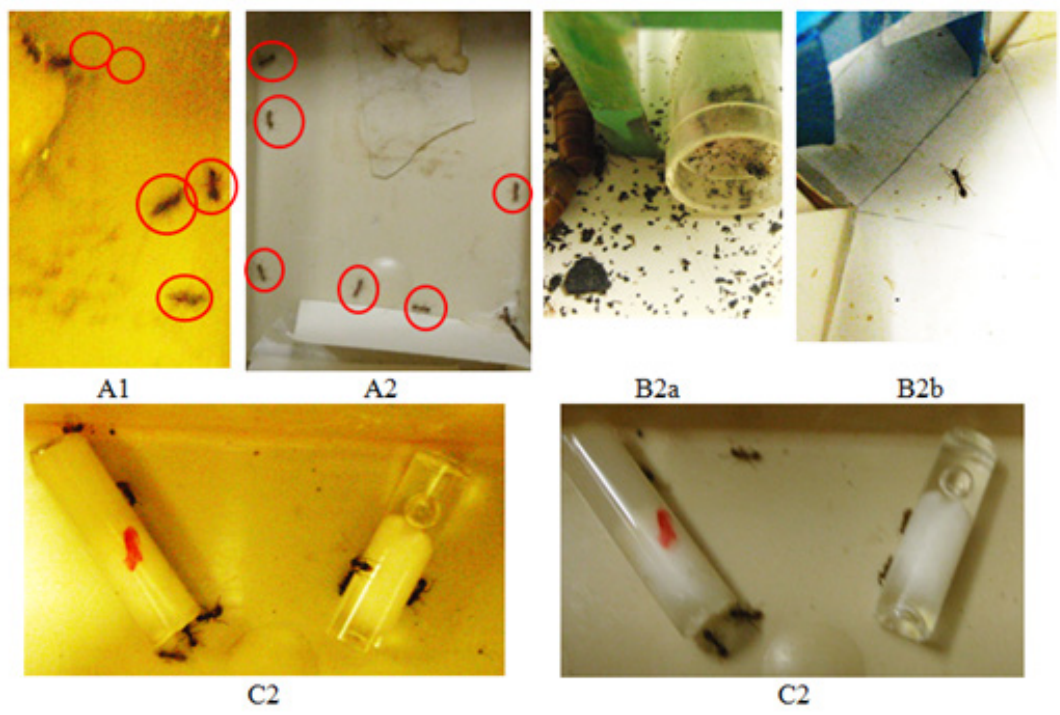


Figure 3. Views of experiments relative to ants' cognition (A), conditioning acquisition (B) and dependence on cashew nuts (C). 1: ants under normal diet; 2: ants consuming cashew nuts. A: whatever their diet, ants could cross a twist and turns path, 5 – 6 ones being beyond this passage; B2 a: ants' training; B2 b: an ant giving the correct response when tested in a Y-maze; C2: ants presenting preference for the solution containing cashew nuts (red dots).

Potential dependence on cashew nuts consumption

The result, presented in Tab. 5, lower part and Fig. 3 C2, showed that ants presented some obvious dependence on cashew nuts consumption. Indeed, ants of colony A as well as of colony B made 42 visits to the tube containing cashew nuts and 12 ones to the tube containing pure sugar water. The total of these numbers (84, 24) statistically differed from those expected if the ants equally visited the two kinds of tube (52, 52) ($\chi 2 = 16.13$, df = 1, $P < 0.001$). This corresponded to 77.78 % of ants choosing the tube containing cashew nuts. An explanation of this dependance was deduced from the study of the decrease of the effect of cashew nuts after weaning (see the following experiment).

Decrease of the effect of cashew nuts after weaning

Numerical and statistical results are given in Tab. 6; they are illustrated in Fig. 4. Briefly, the effect of cashew nuts consumption rather rapidly decreased in 20 – 21 hours, with two rapids loss of activity all over this rather rapid decrease. In details, soon as 3 hours after weaning, the effect of cashew nuts became slightly different from its initial ones, and six hours after weaning, it became highly different. After this quick decrease, the effect of cashew nuts more slowly and linearly decreased, being slightly different from its initial ones, then highly different 12, 15 and 18 hours after weaning. Doing so, it lost about 1/3 of its values. Then, since 18 to 20 – 21 hours after weaning, the activity of cashew nuts again rapidly decreased (from 134 to 95 ang. deg.), 95 ang. deg. being a value similar to the control situation.

Compared with the control situation, the effect of cashew nuts stayed statistically different from the control situation until 18 hours after weaning. Then, it very rapidly decreased and reached the control value at 21 as well as at 25 hours after weaning. This was the second quick decrease of the cashew nuts effect.

The two rapid decrease of the effect of cashew nuts could be perceived by consumer and induced them to take some more amount of this nut, what lead to dependence and may accentuate the side effect of cashew nuts.

Table 6. Decrease of the effect of cashew nuts after weaning. The table gives the values of angular speed obtained over time and the statistical evaluation. The nuts rapidly lost its effect (in about 20 hours) with two quick decreases, one from 0 to 3 hours, the second from 18 to 20 hours. These results are also graphically represented in Figure 4.

Time (hours) after weaning	Angular speed (ang.deg./cm) Median (quartile)	*vs* t = 0			statistics *vs* control		
		$\chi 2$	df	P	$\chi 2$	df	P
0 hs	224 (202 – 232)		-		80	1	< 0.001
3 hs	191 (182 – 232)	10.41	1	< 0.01	48.03	1	< 0.001
6 hs	190 (166 – 206)	12.37	1	< 0.001	48.03	1	< 0.001
9 hs	184 (164 – 218)	10.41	1	< 0.01	35.83	1	< 0.001
12 hs	166 (151 – 203)	14.54	1	< 0.001	32.55	1	< 0.001
15 hs	157 (119 – 163)	25.85	1	< 0.001	25.48	1	< 0.001
18 hs	134 (116 – 175)	25.85	1	< 0.001	18.04	1	< 0.001
21 hs	95 (84 – 126)	80	1	< 0.001	0.83	1	< 0.90
25 hs	96 (88 – 110)	80	1	< 0.001	1.59	1	0.2<P<0.3
control	99 (87 – 118)	80	1	< 0.001		-	

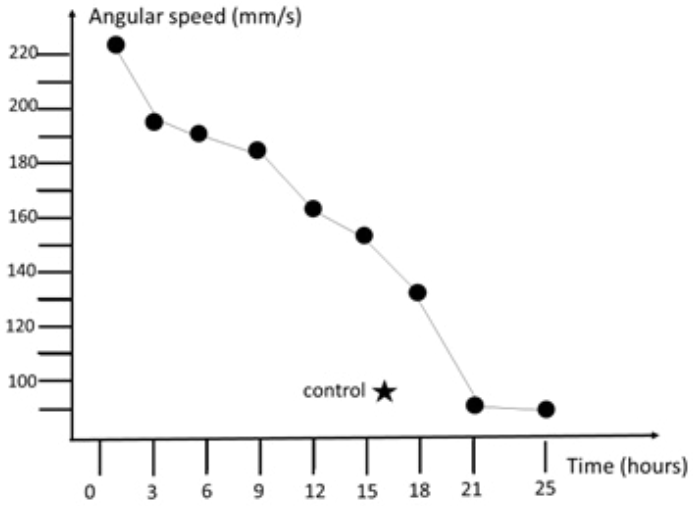
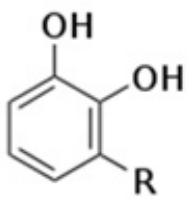


Figure 4. Decrease of the effect of cashew nuts after weaning.

Numerical and statistical results are given in Table 6. Briefly, the effect of these nuts rapidly decreased in about 20 hours, with a rapid decrease from 0 to 3 hours and one from 18 to 20 hours after weaning, what accounted for the development of a dependence on these nuts' consumption, a novel never reported side effect of this fruit.

DISCUSSION AND CONCLUSION

Cashew nuts detain several advantageous dietetics properties, but unfortunately, produce toxic substances which can cause severe dermatitis. Working on ants as models, we confirmed that eating cashew nuts has no detrimental effects, on the contrary, though enhancing the ants' health, they increased their nervousness, excitation and stress. We also revealed a not yet known side effect of these nuts: they lead to dependence (addiction). This is due to the rapid decrease of the effect of cashew nuts after weaning, with over this decreases, two even more quick decrease. As constantly observed, such a kind of decrease lead to dependence [28]. Becoming dependent on cashew nuts, humans will over time consume more and more of this nut, and doing so will increase their probability to develop dermatitis. Figure 5 (upper part) shows dermatitis induced by cashew nuts, and the molecules causing such illness, as well as (lower part) the consequence of the decrease of the effect of these nuts and of the resulting humans' behavior. A precise description of the dermatitis due to the ingestion of or to the contact with cashew nuts has been made by Cisse et al [29]. An informative review about the allergy of cashew nuts has been published by van der Walk et al. [30]. More information can be found in the well documented internet site 'Wikipedia'.



R = $(CH_2)_{14}CH_3$ ou
R = $(CH_2)_7CH=CH(CH_2)_5CH_3$ ou].
R = $(CH_2)_7CH=CHCH_2CH=CH(CH_2)_2CH_3$ ou
R = $(CH_2)_7CH=CHCH_2CH=CHCH=CHCH_3$ ou
R = $(CH_2)_7CH=CHCH_2CH=CHCH_2CH=CH_2$ ou autres

Due to the quick decrease of the effect of cashew nuts, with two very quick one, this nut leads to dependence and so accentuates the possibility of suffering from dermatitis

Figure 5. Upper part: dermatitis induced by cashew nuts; the molecules causing such dermatitis. Lower part: the consequence, never revealed, found thanks to the present experimental work.

Before concluding and reporting a solution to the 'cashew nuts problem', we want to note that our kinds of experiments had the ability to reveal the biological effects of a product (here of cashew nuts) on physiological and ethological traits. These experiments concerned the individuals' locomotion, audacity, tactile perception, social relationships, stress, cognition, learning and memory, adaptation, dependence, and decrease of the effects after weaning. Let us add that fruits other than cashew nuts also contain urushiol; we intend to examine their biological effect.

To conclude, due to their potentially danger of causing dermatitis, cashew nuts should be consumed in moderate amount (i.e., maximally 25 gr per day), and preferably together with other kind of nuts. Let us point out that cashew nuts must not be excluded; they contain many elements excellent for the health. Simply let us use this nut with adequate moderation.

ACKNOWLEDGMENTS

None.

CONFLICT OF INTEREST

I declare having no conflict of interest as for the use of cashew nuts.

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