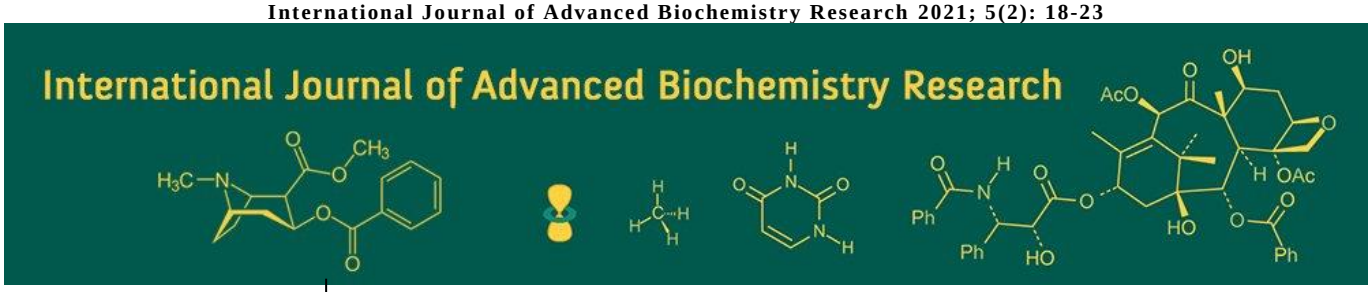

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Comparative study on effect of aluminium phosphide on some nutrients and anti-nutritional factors in *Arachis hypogaea*

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Abstract
The nutrients and anti-nutrients of freshly harvested *Arachis hypogaea* aluminium phosphide preserved (APP) non-aluminium phosphide preserved (NAPP) were investigated. The study aimed at determining the effect of aluminium phosphide on nutrients and anti-nutrients levels of legumes using *A. hypogaea* as a case study. The proximate composition, minerals profile and anti-nutrients were estimated using standard analytical methods. The proximate compositions showed that NAPP is significantly (p<0.05) higher than APP except the fiber (03.01±0.06 mg/100 g; 03.80±0.06 mg/100 g) and ash (01.00±0.06 mg/100 g; 02.23±0.06 mg/100 g) carbohydrate (07.40±0.06 mg/100 g; 03.48±0.06 mg/100 g), protein (19.93±0.06 mg/100 g; 14.94±0.06 mg/100), fat (74.60±0.06 mg/100 g; 68.08±2.75 mg/100 g), and moisture content (06.40±0.06 mg/100 g; 3.00±0.06 mg/100 g). Exception of phosphorus (65.00±0.06 mg/100 g; 63.00±0.06 mg/100 g), the minerals concentration was significantly (p<0.05) higher in NAPP compared to APP. Iron (76.20±0.06 mg/100 g; 62.00±0.06 mg/100 g), potassium (38.01±0.06 mg/100 g; 26.20±0.06 mg/100 g), manganese (26.02±0.06 mg/100 g; 15.10±0.06 mg/100 g), magnesium (11.00±0.06 mg/100 g; 06.00±0.06 mg/100 g), calcium (82.10±0.06 mg/100 g; 72.00±0.06 mg/100 g) and zinc (25.01±0.06 mg/100 g; 14.01±0.06 mg/100 g).The anti-nutritional factors showed significant difference (p<0.05) higher in tannin (9.33±0.69 mg/100 g; 1.90±0.02 mg/100 g), oxalate (32.50±0.60 mg/100 g; 42.50±0.60 mg/100 g), phytate (16.72±0.60 mg/100 g; 5.48±0.60 mg/100 g), alkaloid (16.72±0.6 mg/100 g; 5.48±0.60 mg/100 g) and saponins (40.74±0.60 mg/100 g; 38.20±0.60 mg/100 g) except cyanide (0.02±0.00 mg/100 g; 0.02±0.00 mg/100 g). The study showed that aluminium phosphide negatively affected the nutritional profile of *A. hypogaea*. Thus, the effect of aluminium phosphide should be further investigated *in vivo*.

Keywords: *Arachis hypogaea*, food preservatives, aluminium phosphide, anti-nutrients

1. Introduction
Legumes occupy an important place in human nutrition and are considered as poor man's meat, especially by those who are in developing countries. This is due to the reason that legumes are a good source of protein and slowly digestible carbohydrates [1]. They are very important in human and animal nutrition. Ideally, the basic protein requirement is met by consuming proteins of plant and animal origin. Above all these facts, legumes contain more protein than any other plant proteins. They also have a unique property of maintaining and restoring soil fertility. Legumes are a rich source of nutrients such as protein, starch, minerals and vitamins and also have important health protective compounds (phenolics, inositol phosphates and antioxidants) [2]. This advantageous composition of legume seeds, not only makes them a meat replacer for vegetarians but also as a component of rational nourishment. They serve as a low-cost protein to meet the needs of a large section of the people. However, several anti-nutritional factors present in legume seeds are a major limiting factor for the increased consumption of legumes, whose presence degrades the nutritive value of legumes. This may even lead to health problems which could eventually become fatal to humans and animals if taken in larger amount. In spite of the increasing interest concerning cultivation of pulses, the growth in area and production of seeds, and their application is relatively small (Kozlowska *et al*., 1998) [19].
Legumes contain a wide variety of anti-nutritional factors, such as raffinose family oligosaccharides (RFO's), neurotoxin, proteinaceous compounds, lectins, goitrogenic factor,

amylase inhibitors, and phytic acid (Maia *et al*., 2000; Preet and Punia, 2000; Enneking, 2011) [21, 26, 10]. Food processing methods including soaking (Frias *et al*., 2000; Vidal-Valverde *et al*., 2002) [12, 34] germination, decortications, fermentations, cooking and addition of enzymes have been suggested to reduce the concentration of anti-nutritional factors in pulses which greatly influence their nutritive values. Cowpeas are highly susceptible to pest infestation, and this leads to huge post-harvest losses, lower food quality and poor food safety. To mitigate these losses, the majority of farmers and grain merchants employ various insect control measures, including the use of chemicals not minding the consequences of their actions. The use of chemicals for crop preservation has called the attention of individuals, government agencies and organisations to food quality and safety in the country.

Food preservation is used since ancient times. Food preservatives become an essential thing nowadays, this plays an important role in food transportation. Preservatives are the substances, which are used to prevent food spoilage from microorganisms. This will preserve the food for a long duration from the spoilage (Norkulova, 2016) [23]. Food is an essential thing for human survival. Except our own garden plants, all the food used today has some preservatives. Recently, several microbial provoked teas got noticed in the Western place, probably not only because of trade expansions between west and china, but also because of several health beneficial claims associated with microbial fermented tea (Tarkhasi, 2016; Reena *et al*., 2016; Anisa *et al*., 2016; Amal *et al*., 2016; Nazni, and Karuna, 2016) [32, 28, 4, 3, 22].

Preservation may be of any kind but it should be long lasting for preservation of food and it should be value your money (Lourdes *et al*., 2016; Kumar, 2016; Obajuluwa *et al*., 2016; Osakue *et al*., 2016; Rajani *et al*., 2016) [20, 19, 24, 25, 27]. An example of increasing a process would be to inspire fermentation of dairy products with microbes that convert lactose to lactic acid; an example of preventing a process would be stopping the browning on the surface of freshly cut Red Delicious apples using lemon juice or other acidulated water. Propyl and Methyl have been used as an anti-microbial preservatives in foods, drugs and cosmetics for over 50 years (Ahmed *et al*., 2016; Chugh *et al*., 2016; Trivedi *et al*., 2015; Bernardi *et al*., 2015; Khan *et al*., 2015) [1, 8, 33, 5, 17]. There have been several previous safety assessments undertaken on this substance by several agencies, including FAO/WHO, FDA and FEMA (Bhalla *et al*., 2015; Kataoka *et al*., 2015; Rufina *et al*., 2016; Darwish *et al*., 2017; Ahmed, 2017; Khan and Ahmed; Imlak *et al*., 2017) [6, 15, 30, 9, 1, 16].

Presence of anti-nutritional factors which are generated by normal metabolism of species in natural foodstuffs and act to reduce nutrient intake, digestion, absorption and utilization and produces many other adverse effects. Studies are needed, that will provide ample solutions on the effect of preservative (Aluminium phosphide) in *A. hypogaea* also known as peanut. The study aimed at determining the effect of Aluminium phosphide on nutrients and anti-nutrients levels of legumes of *A. hypogaea* as a case study. To determine the effect of aluminium phosphide on anti-nutritional factors, some mineral profiles and proximate analysis of *A. hypogaea*. The results of this study would provide insight on to whether or not a preservative that is basically for storage is used to see its effect in anti-nutrient, whether the preservative reduces or increases the toxic anti-nutrient or altered the proximate composition or the mineral profile.

2. Materials and Methods

2.1 Sample Collection and Study Area

The study was carried out in the Biochemistry and Molecular Department of Nasarawa State University, Keffi.

2.2 Sample Collection and Preparation

The African peanut (*Arachis hypogaea*) seed samples were collected from farms in Keffi, Nigeria.

The foreign particles in the sample were removed by hand picking. The peanut was then pounded, blended and pulverized into fine powder. The fine powder was used for the analysis.

2.3 Analysis of Samples

2.3.1 Determination of Proximate analysis

Moisture Content: Determination of moisture content was carried by the method of (AOAC 930.15, 2000 and ISO 6496. 1999). Dry matter was determined gravimetrically as the residue remaining after drying at 103 °C in a ventilated oven.

Ash Content: Ash content was determined by gravimetric method according to (AOAC 942.05, 2000) using this equation:

$$\% \text{ Ashe} = \frac{W3-W1}{W2-W1} \text{ X } 100$$

Where, W_1 = weight of empty dish (g), W_2 = weight of the dish and sample (g), and W_3 = weight of dish and residue after incineration (g).

Crude Protein: Crude protein was determined using Macro Kjeldahl Method (AOAC 990.03, 2000) using this formula: Percentage nitrogen

$$(\%N) = (Vs\text{-}V_b) \text{ x M (HCl) x 1 x } 14.007/ (W \text{ x } 10)$$

Where, Vs = ml HCl needed to titrate sample, V_b = ml HCl needed for the blank test M (HCl) = molarity of HCl, 1 = the acid factor, 14.0076 = molecular weight of N, 10 = conversion from mg/g to %, W = weight of the sample (g) and % protein = N×F where F is a factor equal to 6.25.

Crude Lipid: The crude lipid was determined using petroleum ether extract (AOAC 920.39. 2000) with the relation % crude fat = $W_3 – W_2$ x $100/W_1$

Where, W_1 = initial sample weight in grams, W_2 = tare weight of the flask in grams, W3 = weight of the flask and fat residue in grams.

Crude Fiber: Crude fiber was determined by filtration method (ISO 6865, 2000) using Percent crude fibre (%CF) = $(W_2 – W_3)$ x $100 / W_1$

Where, W_1 = weight of the sample (g), W_2 = weight of crucible and residue after drying (g), and W_3 = weight of crucible and residue after incineration (g)

Carbohydrate Content: The carbohydrate content was calculated by subtracting the summed up percentage compositions of protein, lipid, fiber, moisture and ash contents from 100 (A.O.A.C., 1990). % C = 100 - (%P +

%F + %A + %W + %Fi)

Where, C = carbohydrates, P = protein, F = fat, A = Ash, W = water, Fi = fiber

2.3.2 Determination of Mineral composition

The mineral analysis was determined in accordance with the method described by (ISO, 1998; A.O.A.C., 2000) [36]. The absorbance of calcium, phosphorus, zinc, potassium, manganese, and magnesium was measured in the solution at 578 nm and 430 nm respectively, using a spectrophotometer against the blank.

2.3.4 Determination of Anti-nutritional factors

Phytate Content: Phytate content was determined using the method described by Haugh and Lantzsch (1993) [35]. Absorbance was read at 519 nm against a blank (distilled water) in a spectrophotometer (Atomic Absorption spectrophotometer –AAS Model SP9) using

$$\% \text{ Phytate} = \frac{au}{as} \times \frac{C}{W} \times \frac{Vf}{Va} \times \frac{100}{1}$$

Where, Au = Absorbance of test sample, as = absorbance of standard solution, C = concentration of standard solution, W = weight of sample used, V_f = total volume of extract, V_a = volume of extract used

Cyanide Content: The cyanide contents of the sample were determined using the method described by Bradbury *et al.* (1985) using

$$\text{HCN (mg/kg)} = 1000 \times 0.05 \times W \times \frac{au}{as}$$

Where, W = Weight of sample, au = absorbance of the test sample, as = absorbance of standard solution

Oxalate Content: Determination of Oxalate was carried out according to AOAC (2005) using

$$\% \text{ oxalate} = \frac{Vt}{Ws} \times Vme \times Titre \times 100$$

Where, V_t = total volume of titrate = 100, W_s = weight of sample = 2g EQU, Vme = volume – mass equivalent.

Tannins Content: Tannins content was determined by Folin Denis colometric method. The tannin content was calculated as

$$\% \text{ Tannins} = \frac{100}{W} \times \frac{au}{as} \times C \times \frac{vt}{va}$$

Where, W = weight of sample, au = absorbance of test sample, as = absorbance of standard tannin solution, C = concentration of standard tannin solution, Vt = total volume of extract, Va = volume of extract analysed.

Saponin Content: Saponin level was done by the double solvent extraction gravimetric method (Harborne, 1973) [13] updated 2018 in Toxicology Laboratory N.V.R.I. Vom, Jos Plateau state, Nigeria using

$$\% \text{ Saponin} = \frac{W2-W1}{W} \times 100$$

Where, W = weight of sample used, W_1 = weight of empty evaporating dish, W_2 = weight of dish + saponin extract

Alkaloids Level: Alkaloids level was determined by the alkaline precipitation gravimetric method (Harborne, 1973) [13] was used. The weight of alkaloid was determined and expressed as a percentage of the sample

$$\% \text{ Alkaloid} = \frac{W2-W1}{Weight\ of\ sample} \times 100$$

Where, W_1 = weight of empty filter paper W_2 = weight of filter paper + alkaloid precipitate

2.4 Statistical Analysis

Descriptive statistics were used to analyse data obtained from test procedures and the mean values were compared using Microsoft Excel 2013 with a significant difference at the 5 % level of confidence (p<0.05).

3. Results

Proximate analysis of the studied sample is shown in the figure below. Results obtained showed a significant difference (p<0.005) in all the nutrients between the unpreserved and preserved samples except for crude fiber (03.01±0.06, 03.80±0.06) and ash (01.23±0.06, 02.00±0.06) respectively. Meanwhile, the levels of crude fat are much higher than other nutrients.

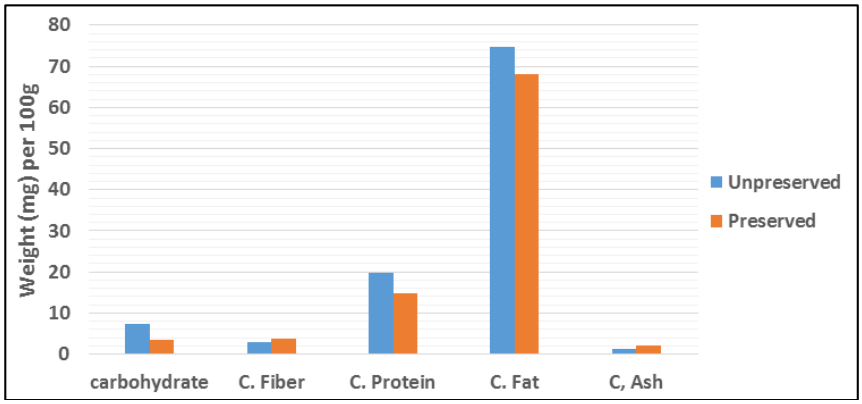

Fig 1: Effect of aluminium phosphide (APP) on proximate composition of *Arachis hypogaea*

Analysis of the mineral profile in the sample is revealed in the chart below. Results obtained showed a significant difference (p<0.005) in all the minerals between the unpreserved and preserved samples except for phosphorus (65.00±0.06, 63.00±0.06) respectively. The unpreserved samples maintained higher levels of minerals than the preserved ones.

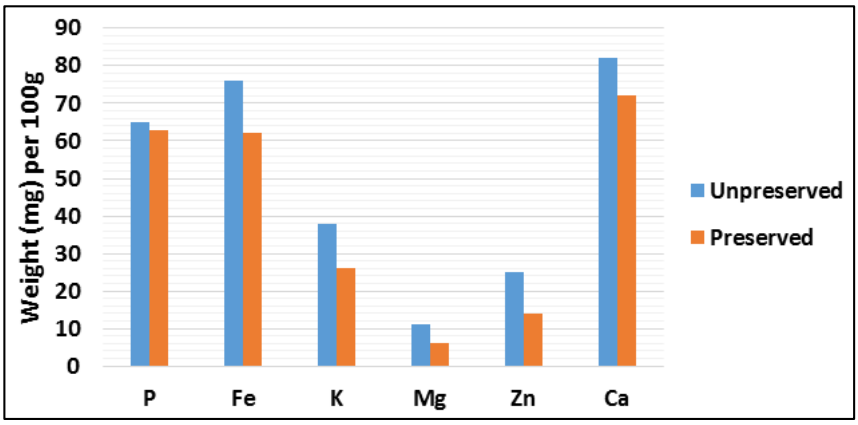

Fig 2: Effect of Aluminium phosphide (APP) on mineral profile of *Arachis hypogaea*

Figure 3 below shows the results of the analysis of anti-nutrients in the analysed sample of *A. hypogaea*. The levels of cyanide in both the preserved and unpreserved samples were infinitesimal. Oxalate, phytate and alkaloid levels showed a significant difference (p<0.005) with an increase in the preserved samples. On the contrary, tannin and saponins levels indicate a decrease in the preserved samples.

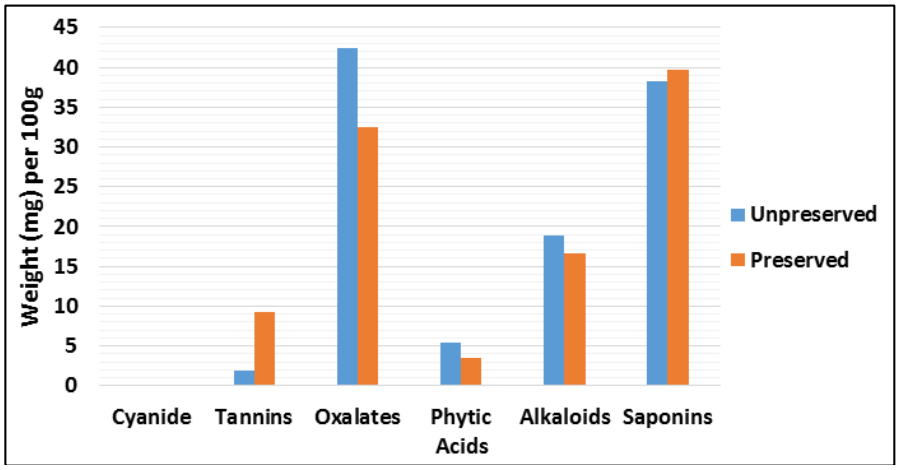

Fig 3: Effect of APP on anti-nutrient factors of *A. hypogaea*

4. Discussion

The proximate analysis of *A. hypogaea* seeds has been studied which showed a significant decrease (p>0.05) in fat, which makes it a suitable source of nutrient that can improve the energy density of man and animals. This is due to the fact that Aluminium phosphide has an effect on fat. The protein in groundnut seeds contributes to the growth and repair of worn-out tissues, will also improve the nutrition of humans and animals. Therefore, Aluminium decreases the nutritional content of protein. There was no significant (p>0.05) difference in the ash content which is relatively low, since the ash contains the minerals which can be estimated from it by atomic absorption spectrophotometry, it can be a good source of nutrients for consumers this shows that Aluminium phosphide does not have any effect on both samples. The crude fibre is not high enough, but can aid digestibility in humans. Aluminium phosphide shows no effect on crude fiber. The carbohydrate content decreases due to the effect of Aluminium phosphide, makes it not suitable for nutrient. Aluminium phosphide decreases the moisture content thereby making it low, this makes the shelf-life to be long and contributes to the stability of *A. hypogaea* and prevent rancidity of the oil.

The mineral content of the peanut showed no significant (p>0.05) difference in phosphorus content which is due to the fact that Aluminium phosphide does not have any effect on both the preserved and preserved. There was a significant (p>0.05) decrease on the preserved sample on iron, potassium, magnesium, zinc, calcium and manganese. This is due to the fact that Aluminium phosphide forms a complex with these ions and thereby reduces bioavailability. Sun-dried groundnut is a good source of magnesium and iron while the roasted groundnut is a good source of potassium, calcium, zinc and phosphorus. The availability of calcium, magnesium, phosphorus is a good indication that the groundnut is so rich in the minerals for bone formation. Calcium is very essential in blood clothing, muscles contraction and in certain enzymes in metabolic processes.

The results of the anti-nutrient contents showed that there was no significant (p>0.05) difference in the cyanide concentration of the Aluminium phosphide on preserved and unpreserved peanuts. The present result suggests that the

cyanide content in *A. hypogaea* is within the permissible level of 200 mg/kg (Richard & Thompson, 1997) [29]. This is probably due to no remarkable difference in the degradation of cyanide (HCN) in the two samples, indicating that preservative might have no effect on cyanide accumulation in the sample. This point to the fact that Aluminium phosphide does not affect cyanide concentration in peanuts. Cyanide when ingested at low concentration is converted to thiocyanide in the body which is less harmful and can be detoxified by the body while accumulation binds to ions in the cytochrome and stops electron transport as a result of oxidative phosphorylation and ATP production is stopped, intracellular oxygen utilization ceases, cell is then forced to use anaerobic metabolism which could lead to lactic acid production and metabolism acidosis. There was a significant (p>0.05) increase in tannins concentration of the preserved but a decrease in unpreserved. This has clearly shown that the release of phosphine gas from Aluminium phosphide (ALP) decreases the tannins content. The analyzed result suggests that oxalate content in all the samples is lower than the permissible level of 250 mg/100 g which cannot induce toxicity in man, but a high level of it above the permissible level can because oxalate is consumed in high amounts so it binds to minerals, vitamins and other nutrient thereby reducing the bioavailability of these nutrient in the body resulting into nutritional problems for example oxalate binds to calcium forming crystals which result to kidney stones. Similarly, the concentration of phytate in both samples is also below tolerance levels of 600-800 mg/100 g but a high amount may result in nutritional problems, such as rickets, and goiter, which is a result of calcium and iodine deficiency respectively. Excessive intake of saponins results in high toxicity due to its haemolytic property, in which it ruptures erythrocytes and releases haemoglobin. It reduces nutrient utilization and conversion efficiency as in ruminant (Cheeke, 1989; Sen *et al*., 1998) [7]. A high level of alkaloids exerts toxicity and adverse effects on humans, especially in physiological and neurological activities.

Table 1: Effect of APP on proximate composition of *A. hypogaea* [Weight (mg) per 100 g]

Compositions	Unpreserved	Preserved
Carbohydrate	07.40±0.06*	03.48±0.06
Crude Fiber	03.01±0.06	03.80±0.06
Crude Protein	19.93±0.06*	14.94±0.06
Crude Fat	74.60±0.06*	68.08±0.06
Crude Ash	01.23±0.06	02.00±0.06
Moisture	06.40±0.06*	03.00±0.06

KEY: Mean ± Standard Deviation. *p<0.05 are considered statistically significant.

Table 2: Effect of Aluminium phosphide on minerals profile of *Arachis hypogaea* [Weight (mg) per 100 grams of sample]

Composition	Unpreserved	Preserved
Phosphorus (P)	65.00±0.06	63.00±0.06
Iron (Fe)	76.20±0.06*	62.00±0.06
Potassium (K)	38.01±0.06*	26.20±0.06
Magnesium (Mg)	11.00±0.06*	06.00±0.06
Zinc (Zn)	25.01±0.06*	14.01±0.06
Calcium (Ca)	82.10±0.06*	72.00±0.06
Manganese (Mn)	26.02±0.06*	15.10±0.06

KEY: Mean ± Standard Deviation. * p<0.05 are considered statistically significant.

Table 3: Effect of Aluminium phosphide on anti-nutrients factors of *A. hypogaea* [Weight (mg) per 100 grams of sample]

Anti-nutrients	Unpreserved	Preserved
Cyanide	0.01733±0.002	0.01633±0.002
Tannins	1.9033±0.015	9.3300±0.690
Oxalates	42.5000±0.600	32.5000±0.600
Phytic Acids	5.48±0.600	3.4200±0.600
Alkaloids	18.880±0.600	16.7200±0.600
Saponins	38.200±0.600	39.740±0.600

KEY: Mean ± Standard Deviation. *p<0.05 are considered statistically significant.

4. Conclusion

The study has demonstrated that artificial preservative also known as Aluminium phosphide (APP) has resulted in a significant (p<0.05) increase in anti-nutrient such as phytic acid, oxalate, tannin, Saponins and alkaloids in leguminous grain (peanut) but no significant increase or decrease in cyanide activity. These results clearly indicate that artificial preservative which is used for storage purposes against insects, microbes and so on is harmful to living organisms as an excess accumulation can lead to metabolic acidosis, respiratory distress syndrome and shock, and there is no specific or effective antidote.

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