# Influence Of Container Nature, Temperature And Duration Of Storage On Fungicide Epoxiconazole Disappearance From Fresh Kolanuts

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**Abstract**: The conservation of fresh kolanuts by farmers in Africa poses a veritable problem occasioning enormous economic loss. Fungicide epoxiconazole has been found as best way to reduce or avoid mould and mycotoxins secretion. In the aim to evaluate the bio-persistence of its residue in kolanuts, the present study has been carried out by treatment of kolanuts with epoxiconazole at concentration of 0.15g/l. Samples for epoxiconazole residue analysis were collected at 0, 7, 15 and 30 days in order to follow the elimination of the pesticide residue. In parallel, the incidence of the temperature and the nature of kolanuts container on the bio-persistence of epoxiconazole reside has evaluated. As results, a rapid elimination of epoxiconazole has been observed but varied following the nature of kolanuts container. After only 7 days of storage, the fungicide was undetected (LD = 0.017mg/kg) with the polyvinyl chloride [PVC] container. The efficiency of traditional container made by leaves of *Thaumatococcus daniellii* has also been demonstrated with disappearance of the residue at 15 days of storage. The third container made by perforated cardboard retarded the residue elimination until 30 days. Temperatures 26 or 29°C have not demonstrated their difference in epoxyconazole residue elimination from kolanuts. Taken together, our results suggested the bio-persistence of epoxiconazole residue in kolanuts after the treatment was very low but influenced by the nature of kolanuts container. In addition to its bio-efficiency previously demonstrated, the fungicide epoxiconazole may be an alternative to improve the conservation of kolanuts by farmers in Côte d'Ivoire.

**Index Terms**: Fungicide-epoxiconazole, bio-persistence-low, Container-nature-influence

## 1 INTRODUCTION

Kolanuts are the cotyledons of some species of Cola, a genus of trees belonging to the family Sterculiaceae [1]. About 40 *Cola* species have been described in West Africa; however, the *Cola* species of economic importance are *C. acuminata* and *C. nitida* [2-5]. *C. nitida* is the most cultivated in Côte d'Ivoire [6] and the major production is intended to exportation in several African countries as fresh kolanuts. African human uses of kolanuts stayed largely traditional uses namely in funerals and ritual sacrifices. In addition, the kolanut is chewed for its alkaloids properties (caffeine, kolanin and theobromine), which dispel sleep, thirst and hunger [7]. Thus, only fresh kolanuts were accepted by consumers with slight preference to white kolanuts over red ones. But, the high moisture in fresh kolanuts estimated at 54-64% [8] enhances its susceptibility to fungus infection during the storage. It appears very important to reduce or avoid fungal infection since that could be consecutive to mycotoxins such as aflatoxins or ochratoxins secretion in kolanuts. Indeed, several fungi species isolated from fresh kolanuts included *Botryodiplodia theobromae, Fusarium pallidoroseum, F. moniliforme, F. cavispermum, F.* oxysporum*, Aspergillus niger, A. fumigatus, A. flavus, A tamarii, A. orchraceus*, *Paecilomyces variotii* and species of *Rhizopus spp.* and *Penicillium* such as *Penicillium funiculosum* [7, 9, 10]. It is well known under some eco-physiological conditions, these fungi species can abundantly produce major mycotoxins aflatoxins, ochratoxins, fumonisins or zearalenone in food. Thus, it had been reported occurrence of aflatoxins and ochratoxins in kolanuts from Nigeria at levels ranged from 5 to 160 ppb for aflatoxins [9] and 0.8 to 65.3 µg/kg for ochratpxin A (OTA) [7]. One way to limit or avoid fungal infection is to use efficient fungicide able to prevent mould proliferation or their mycotoxins secretion. In this perspective, the fungicide epoxiconazole have been studied for its property to prevent mould kolanuts colonization for 9 months of storage. Interestingly, the fungicide epoxiconazole has been found not only to prevent or the proliferation of *Apergillius flavus* which was occurred in negative control but also retarded the contamination of *Penicillium sp*. and OTA secretion until 6 months of storage [11]. But, in parallel to benefit impacts of epoxiconazole in mould and mycotoxins preventing, a bio-persistence of its residues in kolanuts may be considered for the health of consumers. Thus, the present study was to follow the disappearance kinetic of epoxiconazole from kolanuts during the storage. Various containers having used to store the kolanuts, it is also judicious to explore their impact in epoxiconazole bio-persistence. At least, the incidence of temperature has been evaluated.

## 2 MATERIAL AND METHODS

### 2.1 Material

**Fresh kolanuts**: kolanuts were obtained immediately after 5-days curing period from kola merchants at Anyama city in the south of Côte d'Ivoire. The kolanuts samples were collected in a sterile polythene bags, labeled appropriately and assayed *in-vitro* within a week of collection.

**Containers for kolnuts:** Three (3) containers were used namely traditional container i.e. a basket lined with leaves of *Thaumatococcus daniellii* (Benn.) Benth., (Container 1), polyvinyl chloride [PVC], (Container 2) and perforated cardboard (Container 3) containers.

**Chemicals and clean column:** Epoxiconazole (OPAL, 75g/l) obtained from BASF Corporation (USA). Acetonitrile was grade HPLC. The clean column was C 18 column

**Apparatus:** HPLC model used was HPLC Shimadzu LC 10AD VP equipped with Shimadzu RF-10A XL UV detector and a Shimadzu C6R 8A integrator.

### 2.2 Methods

**Kolanuts treatment and storage:** Healthy Kolanuts were firstly collected and abundantly washed by water and then treated by epoxiconazole (OPAL, 75g/l) obtained from BASF Corporation (USA) and diluted in water at concentration of

0.15g/l. Then, fresh Kolanuts were conditioned in various containers such as traditional container i.e. a basket lined with leaves of *Thaumatococcus daniellii* (Benn.) Benth., (Container 1), polyvinyl chloride [PVC], (Container 2) and perforated cardboard (Container 3) containers. All containers were stored at temperatures 26 and 29°C. Samples of kolanuts (2kg) were collected from each container after 0, 7, 15 and 30 days of storage for epoxiconazole analysis by HPLC.

**Extraction, purification, detection and quantification of epoxiconazole**

The method used was based on NF EN ISO 11369 – November 1997 / T 90-123 intended to the quantification of epoxiconazole or other pesticide in water. The method was firstly evaluated according to validation methods such as repeatability, recovery, exactitude of method, limit of detection and quantification. For analysis, 10g of kolanuts sample were put in Waring Blender bowl and 20 ml of water for 2 min. After decanting and centrifugation, all supernatant was loaded at a flow of 1-2ml/min into C18 column which was pre-conditioned by 10 ml of water bi-distilled. Epoxyconazole was slowly eluted by 5 ml of acetonitrile at a rate of 1-2 drops/s. After stirring, analysis was performed by HPLC as previously described and carried out in an isocratic mode using UV detection at wavelengths of 205nm. The mobile phase was a mixture of acetonitrile/water (85:15) and the stationary phase was a Zorbax C18 ODS 2.5mm (25cm x 4.6 mm) column equipped with a pre-column C 18. The peak of epoxiconazole was quantified by measuring the peak area.

### 2.3 Statistical analysis

Data were expressed as mean SEM. The occurrence of epoxiconazole in samples following nature containers, temperature and duration of storage was compared using a Wilcoxom matchedpair test. Statistical significance was assumed at $p < 0.05$.

## 3 RESULTS

### 3.1 Evaluation of the analytical method

The method ensured very good recovery in our study at each spiking level. Three different samples spiked with epoxyconazole at 1, 2 or 5mg/kg were analyzed on the same day. The limit of detection (LOD) and limit of quantification (LOQ) were 0.017 and 0.06 mg/kg, respectively. The average recoveries were 92.16±0.34, 92.1±0.85 and 92.4±1.21% for epoxiconazole levels of 1, 2 and 5 mg/kg, respectively, with n¼3 at each level. Recoveries were very consistent and RSDs were lower than 3%, which demonstrates the precision of the analytical procedure. The method can be viewed as valid according to Directive 2002/26/CE, which indicates that recoveries are acceptable within the range 70–110%. All data were corrected according to the overall recovery (92.38±1.2%). When constructing dose–response curves for epoxyconazole analysis, solutions containing 0.5, 1, 2 and 5mg/kg were measured and the coefficient of linearity ($r^2$) was 0.9995. The data related to recovery, relative standard deviations (RSDs) and range are displayed in Table 1.

**TABLE 1**
Data related to recovery and relative standard deviations (RSD), global recovery was 92.22±0.8%

| Epoxiconazole added (mg/kg) | Epoxiconazole measured (mg/kg) | Recovery % | Recovery average | RSD % |
|---|---|---|---|---|
| 1 | 0.9252 | 92.52 | 92.16±0.34 | 0.70 |
| | 0.9184 | 91.84 | | |
| | 0.9212 | 92.12 | | |
| 2 | 1.84 | 92 | 92.1±0.85 | 0.96 |
| | 1.826 | 91.3 | | |
| | 1.861 | 93 | | |
| 5 | 4.66 | 93.2 | 92.4±1.21 | 1.03 |
| | 4.651 | 93 | | |
| | 4.552 | 91 | | |

### 3.2 Incidence kolanuts container nature on bio-persistence of epoxiconazole

Fig 1 showed occurrence of epoxiconazole after 7 days of storage following to the nature of kolanuts containers. Container 1 was basket lined with leaves of *Thaumatococcus daniellii* (Benn.) Benth., showed low level of epoxiconazole (0.132 mg/kg of kolanuts) while container 3 was not suitable for fungicide elimination with level of 1.48mg/kg. Container 2, made by polyvinyl chloride [PVC] was clearly the best container for elimination of epoxyconazole with only 0.063mg/kg after 7 days of storage.

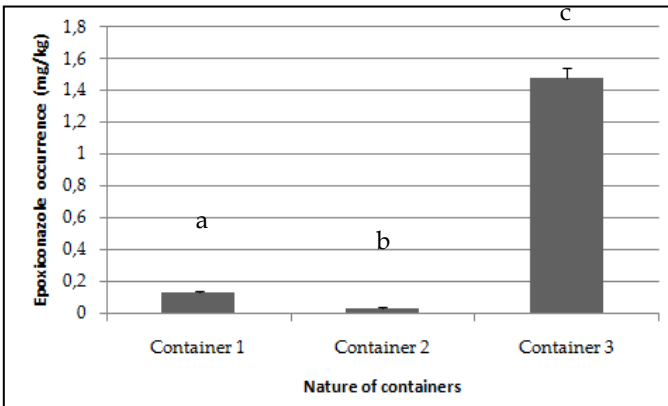


**Fig. 1.** Epoxyconazole elimination from kolanuts following the nature of containers at 7 days of storage. Container 1 was basket lined with leaves of *Thaumatococcus daniellii* (Benn.) Benth., Container 2 was polyvinyl chloride [PVC] and Container 3, perforated cardboard; (a), (b) and (c) explained the significant difference between levels of fungicide showed by the three containers with *p< 0.05*.

### 3.3 Incidence of duration of kolanuts storage on epoxiconazole bio-persistence

Fig 2 revealed incidence of duration of storage on kinetic disappearance of epoxiconazole from kolanuts which was

quick with PVC container and traditional container made by leaves of *Thaumatococcus daniellii*.

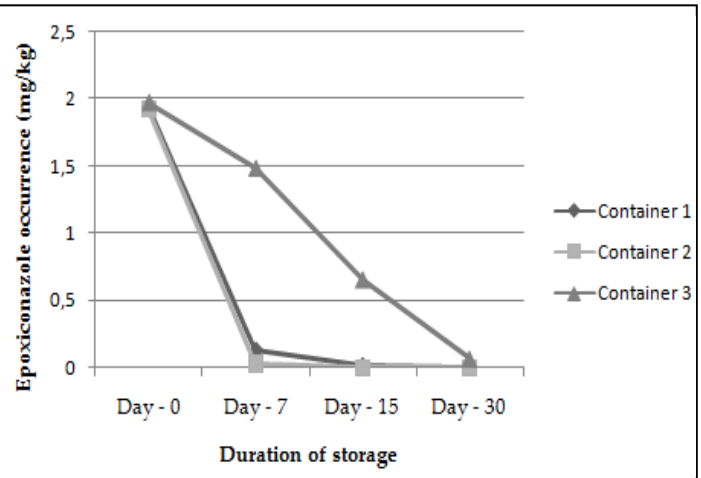


**Fig. 2.** Relative elimination of Epoxyconazole from kolanuts during their storage for each container. After 0, 7, 15 and 30 days of storage.

### 3.3 Incidence of temperatureof kolanuts storage on epoxiconazole bio-persistence

The values of temperature used in the present study were 26°C and 29°C. The container used was only perforated cardboard in which the fungicide disappeared slowly. Fig 3 revealed levels of epoxiconazole at day 7 of kolanuts storage. When compared epoxiconazole elimination levels from kolanuts in both conditions of temperature, there was no significant difference. At temperature of 25°C, the percentage of fungicide elimination

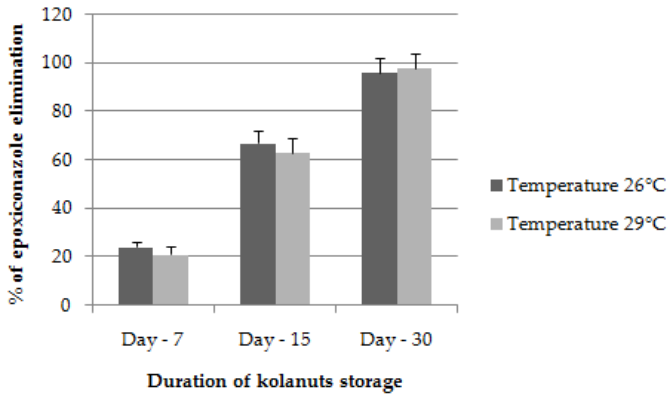


**Fig. 2.** Percentage (%) of Epoxyconazole disappearance from kolanuts under the effect of temperature 26°C and 29°C. Results were not significantly different.

## 4 Discussion

The fungicide epoxiconazole has been found in our previous study to prevent fungal proliferation and mycotoxins secretion in fresh kolanuts in storage [11]. Despite the positive incidence of epoxiconazole in kolanuts storage, a high bio-persistence of its residue could be disqualified it as an alternative for best kolanuts conservation by farmers. For pesticides, there are safety standards namely maximum residue limits (MRL) in order to protect human or animal from harmful levels of pesticides on food. Thus, the present study had been carried out to evaluate the duration of epoxiconazole elimination from kolanuts after the treatment. In parallel, the incidence of temperature and nature of kolanuts containers were also evaluated. As results, it had been demonstrated rapid elimination of the fungicide epoxiconazole from kolanuts when considered all conditions of kolanuts storage. Indeed, 7 days after the fungicide treatment, the residue was undetectable in PVC container (LOD = 0.017mg/kg), 15 days for traditional container made with leaves of *Thaumatococcus daniellii* and 30 days for perforated cardboard. There is an advantage to use traditional container largely used by farmers in Côte d'Ivoire. However, PVC container has been found as best alternative. Indeed, our previous study have demonstrated moulds proliferation and OTA secretion were retarded in kolanuts stored in PVC container when compared to traditional container made with leaves of *Thaumatococcus daniellii* [xx]. At contrast, the perforated cardboard had not been found as suitable container for kolanuts storage for a long period. After, 6 months of storage, OTA had been found at concentrations higher than 2µ/kg which were alarming [12-16] since fresh kolanuts were directly consumed by the African population without serious treatment post-storage [7, 8]. In addition, the present study revealed elimination slowly of epoxiconazole residue from kolanuts stored in perforated cardboard container. Moreover, epoxiconazole residue was not detected after 7, 15 or 30 days (LOD = 0.017mg/kg) in kolanuts stored in all three containers suggested an absence of health risk linked to the fungicide. When we considered a daily consumption of 200g of kolanuts and occurrence of epoxiconazole at level of 0.017mg/kg, the amount of fungicide residue daily intake could estimated at 0.0034mg. Thus, when considered chronic exposition of epoxiconazole for human with weight of 60kg, the daily intake calculated was $5.67 \times 10^{-5}$ mg/kg/day. That was very low to cause disease after long period of kolanuts daily consumption.

## 4 Conclusion

The present study was pioneer in the use of epoxiconazole as fungicide for fresh kolanuts in storage during several months. Epoxiconazole disappeared from kolanuts only 7, 15 and 30 days when used containers namely PVC container, leaves of *Thaumatococcus daniellii* and perforated cardboard respectively indicating real impact of the nature of container on epoxiconazole residue kinetic elimination. The temperatures 26°C and 29°C have not revealed any difference in epoxiconazole disappearance. The fungicide epoxiconazole as revealed previously for its bio-efficiency and now for its absence of bio-persistence could be credible way for best conservation of fresh kolanuts by farmers in Africa.

## Acknowledgment

This research was supported by grant from the FIRCA (Fonds interprofessionnel pour la recherche et conseil agricoles)-Conservation des noix de cola en Côte d'Ivoire.

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