

Pesticide residues in botanics used in feed additives: focusing on wild vs cultivable plants

Fagnon Mahougnon Siméon¹, Araujo Coralie¹, Leguay Clara¹, Hurtaud Johann¹, Kerros Sylvain¹

¹PHYTOSYNTHESE

57 Avenue Jean Jaurès, 63200 MOZAC France

simeon.fagnon@phytosynthese.com; coralie.araujo@phytosynthese.com; clara.leguay@phytosynthese.com; johann.hurtaud@phytosynthese.com; sylvain.kerros@phytosynthese.com

Abstract - Dietary inclusion of herbal components in animal feed is gaining interest due to the reduction of some antibiotic use to decrease drug resistance. Obtaining such products relies on their culture or gathering in a wild environment. Nowadays, pesticide use in agriculture is increasing despite different concerns about public health. The present study provides a pesticide residue assessment of herbal components dedicated to feed additive production. A total of 92 samples of different herbal components were analyzed by three private accredited institutions, PRIMORIS (Belgium), PHYTOCONTROL (France) and EUROFINS (France). These analyses were performed by using gas chromatography tandem mass spectrometry (GC-MS/MS) and liquid chromatography tandem mass spectrometry (LC-MS/MS) methods. Data revealed the presence of residues in 63% of the samples with 10% more than the European Maximum Residue Levels (MRLs). Both herbal components, from wild or culture systems, were contaminated in our samples, respectively 65% and 60%. Wild plants from preserved areas such as the Amazonia forest were found to be surprisingly contaminated. In addition to the detection of pesticides in all countries investigated from various continents, 45% of pesticides were not approved by the European Union Commission. This study provides useful information about plant-based additives by giving awareness to all companies involved in this activity. Despite the low incorporation rate of these additives in feed, a regular monitoring strategy should be developed within each company to ensure safe food for consumers at the top level of the food chain.

Keywords: Pesticide residues, herbal components, feed additives, livestock, European regulation.

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Date Received: 2019-04-17
Date Accepted: 2019-10-17
Date Published: 2019-12-17

1. Introduction

The livestock sector has been actively developed during recent decades at an unprecedented pace over the world [1]. One of the significant innovations in this sector so far is the use of feed additives to support the booming development of livestock production. Feed additives are defined as products that are used in animal nutrition to improve animal performance, feed intake, feed safety and the efficiency of feed utilization for healthy and economic livestock production [2].

Among the various range of feed additives available in the market, botanical components are increasingly used in animal production these recent years [3]. They are commonly regarded as natural alternative solutions after the ban of the use of certain antibiotics and harmful chemical products [4] and [5]. These additives are derived from herbs, spices or medicinal plants directly harvestable from the wild environment or cultivable area.

However, it is widely known that the use of plant protection products (known as pesticides) in agriculture is of common sense for obtaining a high yield. Besides their valuable effects in agriculture, these products can lead to a harmful toxicity to consumers through their residues [6] and [7]. Pesticide residues are the deposits of a pesticide active ingredient, its metabolites or breakdown products present in some components of the environment after its application, spillage or dumping [8]. They are present in various agricultural models with a potential risk to animal and human health from their exposure to primary and derived agricultural products and also plant extracts, essential oils and so on. Moreover, wildlife (flora and fauna) could be unintentionally exposed to pesticides residues by drift

from nearby agricultural crops. Both animals and humans are vulnerable to pesticides residues through their contamination from inhalation of contaminated air or dietary intake of contaminated food [9]. Therefore, concern has been raised by many organizations and countries over the potential accumulation of pesticides in tissues of livestock animals that serve as food for humans [10] and [11].

In order to promote and protect the health of people and communities, regulatory authorities have established maximum residue limits (MRLs) authorized into a commodity. The MRL is the maximum concentration of a pesticide residue (expressed as mg/kg), recommended by the European Union pesticides regulation to be legally permitted in food commodities and animal feeds [12]. In such a trend in which livestock are supplemented with botanical derived products, the risk of cross contamination should not be underestimated. Thus, herbal feed additive companies need to develop a monitoring strategy to prevent consumers from subsequent contamination which may definitely lead to chronic toxicity. PHYTOSYNTHESE is a French company that proposes plant extract-based feed additives for livestock (poultry, ruminant, swine, fish and shrimp). Because of continuous use of different plant species from various countries around the world, this company has implemented a strategic method of pesticide residue evaluation in ingredients dedicated to animal feed additives. The objective of this study was to assess the probable contamination of various botanical ingredients (wild or cultivable) intended to be used for feed additive production during the year 2017.

2. Material and Methods

2.1. Sampling

Herbal components sampled for this analysis were purchased from various companies around the world. Boldo powder (*Peumus boldus*), cat's claw powder (*Uncaria tomentosa*), betel nut powder (*Areca catechu*), lemongrass essential oil (*Cymbopogon citratus*) and wheat middlings powder (*Triticum sp.*) respectively originated from Chili (Andean precordillera), Peru (Amazonian area), Indonesia, India and France. Sophora powder (*Sophora japonica*), eucalyptus essential oil (*Eucalyptus globulus*) and siberian ginseng powder (*Eleutherococcus senticosus*) were collected from China. Turmeric powder (*Curcuma longa*) came from China, India, Mexico and Sri Lanka. Moreover, thyme powder (*Thymus vulgaris*) was collected from Egypt, Morocco

and Poland. Ajowan essential oil (*Carum ajowan*) were purchased from India. Cassia powder (*Cinnamomum ssp.*) originated from China and Vietnam. Finally, fenugreek powder (*Trigonella foenum-graecum*) came from France, India and Tunisia. Betel nut, boldo, cassia, cat's claw, eucalyptus and sophora were collected in the wild environment while ajowan, fenugreek, lemongrass, siberian ginseng, turmeric and thyme were cultivated by farmers. Wheat middlings are considered as a comparative ingredient with the common plant material used in herbal additive sector. In total, 92 herbal components were sampled for laboratory pesticide detection. A minimum of 100g per sample was individually labelled and packed in a polyethylene closed bottle (essential oil) or bag (powder and dried extracts) and transported to the laboratory in compliance with EU guideline 2002/63 recommendation [13].

2.2. Sample Analysis

Pesticide residue analysis was performed in three European private institutions PRIMORIS (<http://www.primoris-lab.com/fr-fr>), PHYTOCONTROL (<http://www.phytocontrol.com/>) and EUROFINS (<https://www.eurofins.fr/>). These institutions are specialized in pesticide and contaminant residue detection with accreditation ISO 17025 delivered respectively by Belgium Accreditation organization (BELAC) and French Council for Accreditation, Audit and Control (COFRAC).

Unfortunately, the complete analytical methods could not be disclosed in this paper. They consist of chronological steps from herbal component extraction to analyses by gas chromatography tandem mass spectrometry (GC-MS/MS) and liquid chromatography tandem mass spectrometry (LC-MS/MS). This method has the advantage of determining many pesticides in one single run. In addition, the multistage mass spectrometry (MS-MS) is regarded as useful tool for detecting low levels of analytes when coupled with chromatographic techniques [14]. The selected labs follow the Directorate-General for Health and Food Safety (DG SANTE) directives according to the guidance document on analytical quality control and validation procedures for pesticide residues analysis in food and feed [15]. Their know-how is in line with European monitoring program, in which proficiency tests are organized by the European Reference Laboratories for Residues of Pesticides.

2.3. Headings

All detected pesticide values from each sample were compared to the Maximum Residue Level (MRL) defined by European Union (EU) pesticide database according to their category code number. These code numbers were consultable online [6]. Moreover, the ingredients were categorized according to their use in plant-based additives.

3. Results

3.1. Pesticide residues in vegetable ingredients used in feed

Residual pesticide levels for 92 samples of various cultivable and wild herbal components have been assessed. The results showed that pesticide residues were not detected in 34 samples (37%), while 58 samples (63%) showed detected pesticide levels (table 1). From these detected samples, 9 (10%) showed pesticide residues exceeding their European MRLs according to the value established by European Union pesticide database. Data showed that residual pesticides were present at a remarkable percentage in wild herbal components (65% of 52 samples, although it is not less important in cultivable plants (60% of 40 samples).

Moreover, the number of samples showing detected levels of residual pesticides above the adequate MRLs was higher in wild herbal components (13% with 7 samples) whereas cultivable herbal components were at 5% (2 samples) (Figure 1).

All herbal components analyzed showed a high (>80%) percentage of contaminated pesticides except for fenugreek (0%) and relatively siberian ginseng (20%). The highest percentage of detected residual pesticides was obtained in wheat middlings and cassia (100%), sophora (88%), turmeric (77%), thyme (75%) and relatively cat's claw (62%), betel nut (63%) and eucalyptus (60%). Among the cultivable herbal components analyzed in this study, more than 70% of wheat middlings and turmeric samples were detected positive for residual pesticides below their MRLs. In wild harvestable plants, only sophora and betel nut (>60%) showed the similar trend. In wild plant samples, data showed that boldo and cat's claw were identified for positive residual pesticides above their MRLs respectively at 35% and 25 % of their samples. Thyme is the only cultivable herbal component that contained pesticide residues above the MRLs.

Table 1. Origin, form, harvest environment, category code number, number and percentage of herbal components sampled without detected pesticide residues, and pesticide residues detected below and above the European maximum residue limits (MRL); PRD = Pesticide Residue Detected. EO = Essential oil.

Herbal components	Harvest environment	Form	*Category code number	Origin	Number of samples	Not detected	PRD<M RL	PRD>M RL
Ajowan	Culture	EO	0820030-001	India	2	1 (50%)	1 (50%)	0 (0%)
Fenugreek	Culture	Powder	0810080	France	1	1	0	0
				India	2	2	0	0
				Tunisia	1	1	0	0
				Total	4	4 (100%)	0 (0%)	0 (0%)
Lemongrass	Culture	EO	0256100-004	India	5	3 (60%)	2 (40%)	0 (0%)
Siberian ginseng	Culture	Powder	0212990	China	5	4 (80%)	1 (20%)	0 (0%)
Thyme	Culture	Powder	0256070	Egypt	2	0	0	2
				Morocco	2	1	1	0
				Poland	2	0	2	0

				Total	6	1 (17%)	3 (50%)	2 (33%)
Turmeric	Culture	Powder	0840030	China	1	1	0	0
				India	8	1	7	0
				Mexico	2	1	1	0
				Sri Lanka	2	0	2	0
				Total	13	3 (23%)	10 (77%)	0 (0%)
Wheat middlings	Culture	Powder	0500090	France	5	0 (0%)	5 (100%)	0 (0%)
Betel nut	Wild	Powder	0120050-001	Indonesia	8	3 (37%)	5 (63%)	0 (0%)
Boldo	Wild	Powder	0256990	Chile	17	9 (53%)	2 (12%)	6 (35%)
Cassia	Wild	EO	0830010-002	China	3	0	3	0
				Vietnam	3	0	3	0
				Total	6	0 (0%)	6 (100%)	0 (0%)
Cat's claw	Wild	Powder	0830990	Peru	8	3 (38%)	4 (50%)	1 (12%)
Eucalyptus	Wild	EO	0256990	China	5	2 (40%)	3 (60%)	0 (0%)
Sophora	Wild	Powder	0256080-990	China	8	1 (12%)	7 (88%)	0 (0%)
Total					92	34 (37%)	49 (53%)	9 (10%)

* The MRL of each category is consultable online: <http://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=product.selection&language=EN>

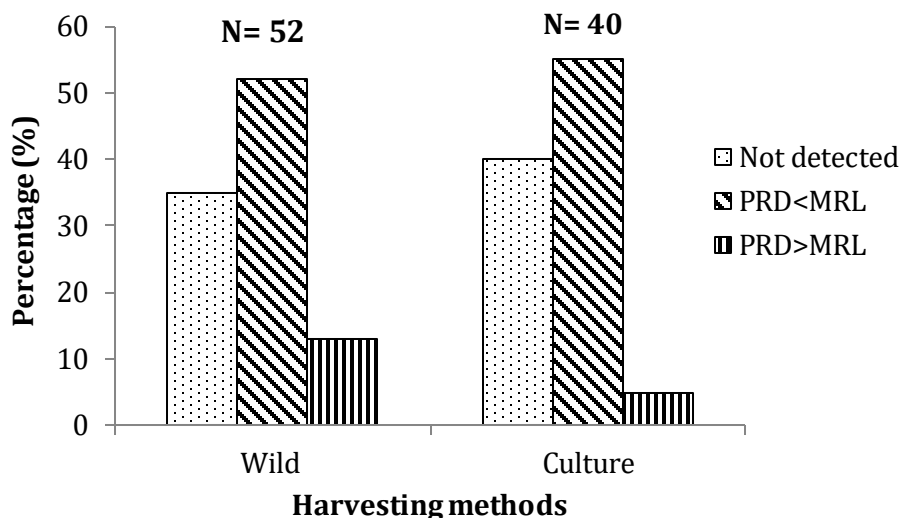


Figure 1. Number of wild and cultivable herbal component samples containing pesticide residues at not detected level, below the European MRL (PRD<MRL) and above the European MRL (PRD>MRL), N = number of samples, PRD= Pesticide Residue Detected.

Herbal components analyzed in the present study come from different countries. The number of herbal component samples containing pesticide residues following their origins at various detected level are shown in figure 2. From a total of 52 samples in Asia, 18 (35%) did not contain pesticide residues at a detectable limit, whereas 34 samples (65%) contained residual pesticides below their adequate MRLs. In Europe, 1 sample (13%) out of 8 analyzed was not detected positive for pesticide residues while 7 samples (87%) contained detectable levels of pesticide residues below the MRLs. None of the samples from Europe and Asia

showed detectable residual pesticides above their specific MRLs. However, herbal components from Africa (2 out of 5 samples) and America (7 out of 27 samples) revealed the presence of pesticide residues above their MRLs. In Africa, the presence of high detectable levels of residual pesticides observed is attributed to the 2 samples of thyme powder purchased in Egypt. 6 out of 17 samples of boldo (35%) from Chile (South America) were highly positive for residual pesticides (>MRLs). In Peru (South America), 1 sample of cat's claw out of 8 (12%) contained residual pesticides at a level exceeding their MRLs.

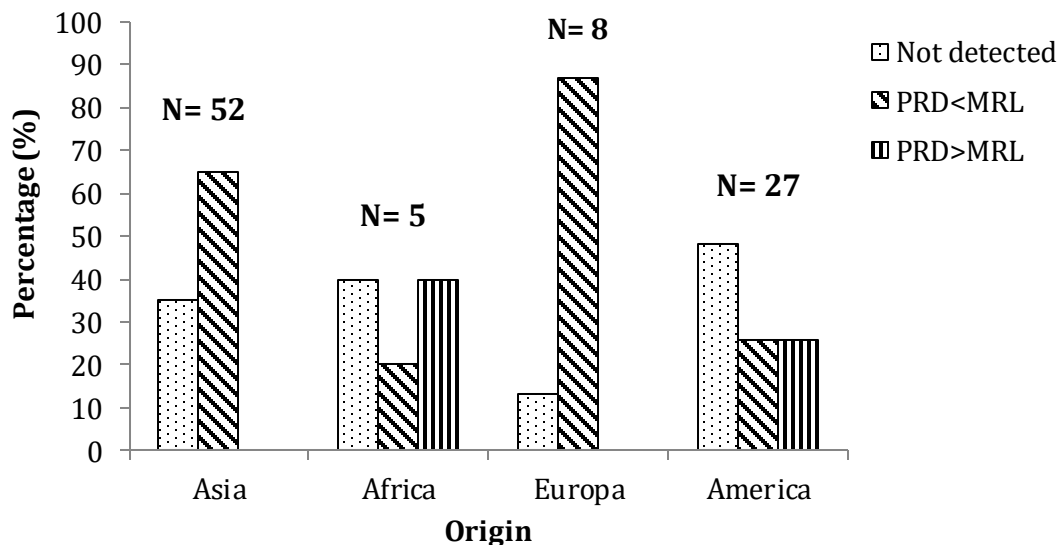


Figure 2. Percentage of herbal component samples containing pesticide residues following their origins at not detected level, below the European MRL (PRD<MRL) and above the European MRL (PRD>MRL), N = number of samples, PRD= Pesticide Residue Detected.

3.2. Detection frequencies of pesticide residues in herbal components

A total of 47 pesticides were determined in 92 herbal component samples from various regions. Out of 46 pesticides detected in this sample, 19 (41%) were identified only once: permethrin, iprodione, quinalphos, prosulfocarb, dicofol, acetochlor, DEET (N, N-diéthyl-3-méthylbenzamide), phorat-oxon and phorat sulfoxide, propamocarb, hexachlorobenzene, dimethoate, profenofos, tetraconazol, thiamethoxam, bifenthrin, fipronyl, carbofuran and flucyثورinate. Chlorpyrifos ethyl (20%), cypermethrin (17%), carbendazim and benomyl (13%), lambda cyhalothrin (8%), gamma cyhalothrin (8%) and anthraquinone (7%) are the residual pesticides frequently detected in the samples. From all these pesticides found in the samples, chloroneb (100% from 4 detections, tetramethrin (100% from 2 detections), rotenone (20% from 5 detections) and gamma cyhalothrin (14% from 7 detections) were detected above European MRLs.

Three categories of pesticides were detected in the analyzed samples. These are insecticides (66%), fungicides (23%) and herbicides (11%). None of the

herbicides were detected above their MRLs. Among pesticides detected above their MRLs, three active insecticide ingredients (gamma cyhalothrin, tetramethrin and rotenone) were identified while the only fungicide active ingredient above the MRL was chloroneb. Gamma cyhalothrin was determined above the MRL within 2 samples of thyme from Egypt while rotenone was identified in cat's claw from Peru. Boldo powder from Chile was the only herbal component that showed two different residual pesticides above their MRLs (4 samples for chloroneb and 2 samples for tetramethrin). In addition, results showed that 45% of pesticides detected were not approved by the European Union Commission.

Figure 3 shows the pesticide active ingredients detected above their MRLs following the harvesting methods of plants (culture vs wild collect). 3 out of 4 pesticides detected above their MRLs (rotenone, tetramethrin and chloroneb with 7 samples) were identified within herbal components from wild population whereas gamma cyhalothrin was detected in cultivable plant (2 samples).

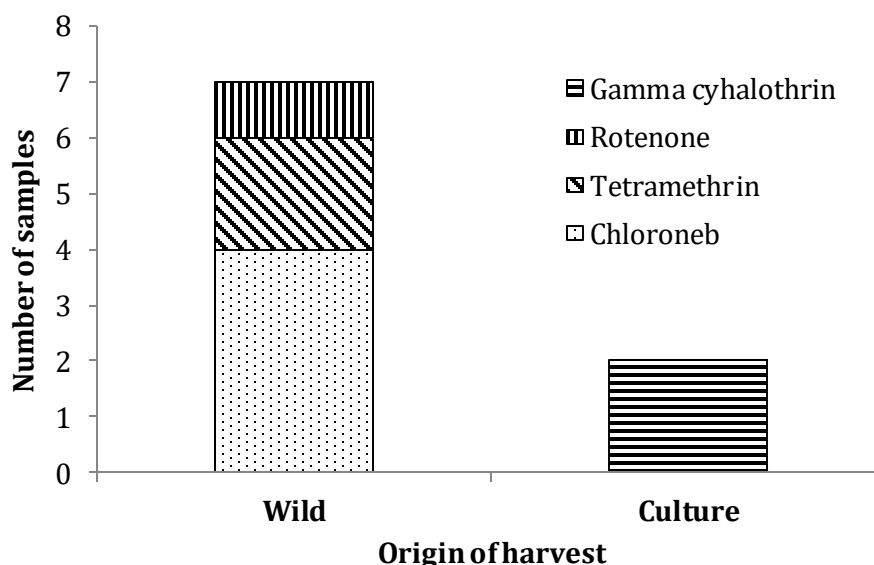


Figure 3. Residual pesticides detected above the European MRL following the harvesting methods of plants.

3.3. Multiple pesticides residues

Figure 4 shows the proportion of herbal components containing no pesticide residue, one and multiple (2, 3, 4 and more than 4) residues. From 92 samples analyzed, 37% did not reveal the presence of at least one residual pesticide. Regarding the number of active ingredients detected per herbal component sample, 25% were contaminated with a single residue

and 13%, 10% and 3% contained 2, 3 and 4 residues, respectively. In addition, 12% of sampled plants showed a contamination with more than four residual pesticides. Wild plant population showed a relatively low proportion of multiple residues contamination. Proportions were similar in some multiple residues such as 2 and 4.

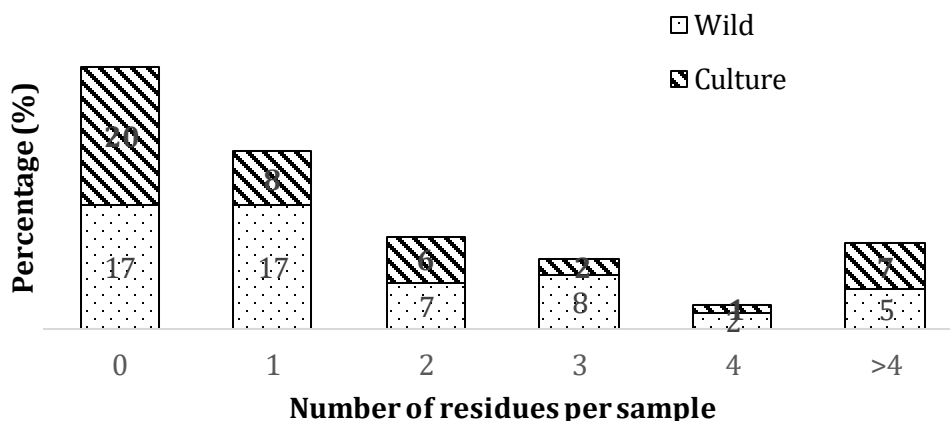


Figure 4. Percentage of pesticide residues detected per sample in herbal components within wild and cultivable groups.

3. Discussion

Residual pesticide analysis of 92 herbal components collected from various regions around the world indicated considerable presence of some residues in investigated samples. Data showed evidence of pesticide contamination in 53% of total samples analyzed among which 10% were above the European MRLs. The presence of residual pesticides in crops is largely noticed by various authors although their level of contamination is always different [16] and [17]. Our results are quite similar (in terms of percentage) to EFSA in which 96.2% (81.482) of analyzed food products were within limits permitted in EU legislation and 50.7% of the tested samples were free of quantifiable residues [18]. This presence of pesticide residues in plants ingredients may be due to the regular use of these chemical substances by farmers with no or little knowledge and education about application dose, methods of application and appropriate intervals between harvesting and pesticide treatment [17]. Surprisingly in our data, both plants from culture and wild environment were detected positive for residual pesticides. Herbal component samples from wild environment (cat's claw powder, sophora powder, betel nut powder, cassia EO and eucalyptus EO) showed greater proportion of contamination (above 50%) except for boldo. However, 35% of boldo samples were contaminated above the European MRLs. This finding highlights a potential passive contamination of some plants harvested from wild environments like Amazonian area and Andean precordillera. It may result from a natural puff of wind or water running from neighbouring area. Despite of the fact that some plants

are harvested from a wild environment, they may be subject to chemical pesticide application such as herbicide during earlier years and insecticide against pest outbreaks like for eucalyptus in Australia [19]. The persistence of such chemical products in the environment or plant derived products is quite variable and may depend on the time or frequency of application and the harvested parts of a plant.

Our study revealed the noticeable detection of pesticide residues in all the investigated continents. This confirms the worldwide trend of pesticide application in agricultural field. Many authors indicated the presence of residual pesticides in edible vegetables and fruits from some countries investigated [20] and [21] even though not in the same plant screened in the present study.

Chlorpyrifos ethyl, cypermethrin, carbendazim and benomyl are the most frequent and common pesticide residuals found in the investigated samples. This means that they are regularly involved in chemical treatment in agriculture for diverse plants and in many countries. However, they were not above the MRLs in the present study. Among residual pesticides detected in our samples, many were not approved by European Union Commission (carbendazim and benomyl, anthraquinone, thiabendazole, chloroneb and so on) because of their potential risk to the applicator and their long-term persistence in the environment. Many countries or regions throughout the world have different pesticide regulations. This discrepancy may explain the variation of pesticide residue category identified from many countries. Pesticide or its amount permitted in one country may not be the same authorized in other countries or regions. In addition, as many pesticides are

released directly to the environment when they are applied, they can move far from their sources of application to the nearby area including wild environment. It could be the case of rotenone, tetramethrin and chloroneb detected in wild harvestable plants in exceeding amounts like those from Andean precordillera and Amazonian area. Moreover, many herbal components sampled (63%) showed the presence of multiple residues. According to Jallow et al. [16], it may be the consequence of application of various pesticides in the same environment or culture along the cultivation period in order to protect crops against different pests and diseases.

Consequently, the use of such a contaminated product such as herbal additives in animal feed could result in deleterious effects for animals and consumers. As livestock have a daily fed diet, if contaminated, residual pesticides may be accumulated in the animal tissue [22]. This consists of a bioaccumulation of chemical substances in the animal products like milk, meat, fat and eggs. Therefore, human beings may be chronically exposed to these persistent substances through dietary intake. Obviously, pesticides are known to cause a wide range of toxic effects and are potential source of severe health risks [23]. Many authors have already revealed the presence of such pesticides in animal products although more in developing countries [9] and [23]. However, that doesn't mean additives are the main route of contamination but it should not be excluded as it was less documented so far. Moreover, the supplementation dose of herbal feed additives in animal nutrition is very low (0-2kg/t of feed) compared to other vegetables like cereals knowing that all wheat middling samples showed pesticide residues. In this case, dilution principle is not applicable to define the difference of MRL between an additive and a feed. They are regulated by the same standard. The dilution level of an additive into feed usually results in undetectable levels of pesticide contamination in the feed (below the limit of quantification). Therefore, companies using herbal components for their additives should continue or enhance their level of pesticide monitoring in order to deliver safe products to consumers. They should give greater priorities to pesticide control in any herbal components without distinction of their source and harvesting or culture methods.

5. Conclusion

The present study investigated the presence of pesticide residues in commonly used herbal components

for feed additives originating from various regions. The results indicated remarkable contamination of herbal components with pesticide residues above the MRLs in some products. Moreover, both plants from wild and culture environments were contaminated with persistent chemical substances. From a health perspective, companies proposing herbal feed additives should play an effective role in implementing relevant risk assessment and regular monitoring strategies to ensure safe products for consumers.

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