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Review

The Spread of Invasive and Poisonous Plants: A Lesson from Alkaloids

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Abstract: Invasive plant species pose a significant threat to global biodiversity and ecosystems. Climate changes favor the spread of non-native plants, whether voluntary or accidentally introduced into a new environment, as these plants possess a greater ability to adapt to changing environments. The spreading of these alien species has a negative impact also on agro-ecosystems, on agricultural yields, and on the nutritional quality of food crops. The high metabolic plasticity of these plants helps them to adapt to new ecosystems, enabling them to succeed in competing with crops. In particular, many alien plants are producers of alkaloids. These molecules represent the main chemical defense to biotic stressors and also the major risk for human health. In this review, we focused on invasive plants producing tropane alkaloids (TAs) and pyrrolizidine alkaloids (PAs). We explored the potential role of these molecules in the fitness of invasive plants in the context of climate change and reviewed the knowledge regarding their biosynthesis steps and examined the mechanism of toxicity when accidentally ingested. Finally, we summarized the most efficient analytical and molecular methods to detect either alkaloid contamination or the presence of invasive plant contaminants, which are the source of these molecules, in food crops. Possible solutions and precautions to ensure food safety have been also proposed.

Keywords: tropane alkaloids; pyrrolizidine alkaloids; alkaloids biosynthesis; climate change; food contaminants; food safety

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1. The Biosynthesis of Plant Bio-Molecules in a Changing Environment

Over millions of years of evolution, plants have evolved a myriad of strategies to protect themselves and to escape from unfavorable conditions. The expansion of the root system, the regulation of stomata activity, the formation of mycorrhizal association, the production of protective proteins, and even a controlled cell death and senescence are just a few examples of strategies they developed over time. Among these adaptive mechanisms, the ability to synthesize a wide range or even specific types of molecules is perhaps the most distinctive trait of the plant kingdom. In some cases, the diversity of these compounds can mirror the phylogenetic diversity of plants by exhibiting specific functions against stress [1]. Moreover, specialized metabolites can accumulate differentially across plant organs, tissues, and cells and their biosynthesis can be located in specific sub-cellular compartments to properly exert their protective function. Due to these general characteristics, the adaptation of a specialized metabolism plays a crucial role in helping plants adjust to changing environments, even more so than other strategies.

Climate changes impact the performances of plants, and this effect is particularly pronounced in domesticated plants. We could generally assert that most domesticated plants

have lost their biochemical plasticity, when compared, for example, to their wild ancestors. The chemical complexity of these molecules means their biosynthesis is energetically costly and occurs at the expense of growth, diverting resources away from plant yield [2]. The trade-off between growth and defense has been the subject of studies for decades. As, for example, reported in maize, where the domestication, although improving growth and yield traits, weakened defense towards insects as compared to its wild parent teosinte [3]. If domesticated plants are often weak in their ability to adapt to multiple stressors, invasive plant species exhibit remarkable resilience and adaptability to new environments that differ significantly from their native ranges. These plants retain the ability to effectively reallocate resources and adjust their defenses as a response to stresses. They can leverage a plethora of specialized metabolites to thrive under changing conditions. Importantly, many of these molecules are toxic to humans, posing potential health risks. This is particularly concerning for invasive species, as these plants are often present among crop cultivations, leading to the risk of cross-contamination and increased exposure to these harmful compounds. It has been estimated that there are over 200,000 specialized metabolites in the plant kingdom. Surely, invasive plant species showed the most incredible variability. Specialized metabolites are classified based on chemical core structures and biosynthetic origin grouping them in phenylpropanoids, N-containing compounds (alkaloids), fatty acid derivatives, benzenoids, terpenes, and glucosinolates [4]. In this review, we will focus on alkaloids from invasive plant species and their impact on food safety. Alkaloids are a group of specialized metabolites showing numerous physiological and pharmacological impacts and utilizations. These metabolites have attracted considerable interest in invasive plants because they have been involved in defense responses against herbivores and pathogens [5]. In the following paragraphs, we will describe the biosynthesis and distribution and the analytical methods for the analysis of alkaloids. In particular, we focus on a class of distinct molecules such as tropane alkaloids (TAs, e.g., scopolamine and hyoscyamine) and pyrrolizidine alkaloids (dehydro PAs) that are the common causes of intoxication from the consumption of plant-derived food products. We aimed to present topics discussed here in a way that is accessible to a wide range of readers, as this review is interdisciplinary in focus.

2. Alkaloids from Invasive Plants: A Successful Strategy for Environmental Adaptation but a Risk for Food Safety

Invasive plant species, also better defined as alien plants, are introduced accidentally or deliberately into places outside their native boundary [6]. Their introduction and dispersion generate negative impacts on biodiversity and related ecosystem services [7]. *Lantana camara* L. (Verbenaceae), *Parthenium hysterophorous* L. (Asteraceae), *Ricinus communis* L. (Euphorbiaceae), and *Ageratum conyzoides* L. (Asteraceae) are just a few examples of invasive plant species. Their great adaptability allows invasive species to thrive under a variety of environmental conditions, making them formidable competitors in new territories. Most of the metabolic weapons of invasive species are committed to biotic defenses, but scarce is the knowledge on their possible role under abiotic stress or concurrent abiotic stresses. A study by Yin et al. [8] examined insect resistance and drought tolerance in populations of *Ambrosia artemisiifolia*, an invasive plant originating from the American continent. The authors observed the ability of this plant to reallocate plant resources in response to the specialist beetle *Ophraela communa* after re-association with this insect. The genetically controlled trade-off resulted in reduced tolerance to drought, an important evolutionary metabolic plasticity probably shared with other invasive plants, which needs to be better scientifically explored. In particular, to date, there are few studies directly comparing PA and TA alkaloid profiles of native and invasive plants of the same species under conditions of abiotic stress. Data coming from these studies can be useful for better understanding the successful invasive behavior, but also to foresee the impact on the safety of food. Climate change may favor the spread of these species into different non-native habitats. Regarding the effects of abiotic stresses imposition on alkaloid biosynthesis, several investigations on medicinal plants have dealt with this topic. To date, Yahyazadeh et al. [9] reported that *Catharanthus*

roseus plants grown under drought stress markedly accumulated higher content of indol alkaloids as compared to well-watered controls. Conversely, the exposure of these plants to high salt concentrations did not affect the alkaloid concentrations which was even lower in relation to the total plant biomass. Moreover, the same authors reported the increase in alkaloids in leaves of *Chelidonium majus* under salt and drought stress. A general possible explanation for the increase in alkaloids upon abiotic stress relies on the fact that plants under adverse conditions promptly close stomata to reduce CO_2 fixation thus inhibiting the chloroplastic electron transport chain [10] and then all reactions consuming NADPH, including alkaloid biosynthesis, could be enhanced. This can possibly explain that alkaloids in abiotic stress might not have a real induction for a biological or ecological role but their increase might be the result of a passive mechanism [11]. The actual lack of knowledge on regulatory mechanisms behind alkaloids biosynthesis even in medicinal plants delays the development of ad hoc metabolic engineering strategies and molecular tools for limiting the accumulation or for temporally and spatially regulating the production of toxic alkaloids in invasive species. Besides the use of molecular tools, several alternative strategies have been established for the management and monitoring of invasive plants to preserve natural biodiversity according to the Aichi Biodiversity Targets https://www.cbd.int/sp/targets (accessed on 15 July 2024). Unfortunately, most of these strategies have been unsuccessful since the number of these plants is continuously growing [12,13]. We would like to point out that this is not only a threat to natural biodiversity and crop loss but, the presence of these plants poses an important problem due to their toxicity.

In particular, the high concentrations of PAs and TAs in these plants, especially if enhanced by environmental changes, can act as contaminants in food and feed, leading to significant health risks. There is global alert and interest regarding these two classes of molecules, to the extent that they were included on the agenda and discussion paper of the last Session of the Codex Committee on Contaminants in Foods (CCCF17).

3. Biosynthesis and Health Implications of Tropane Alkaloids

3.1. Tropane Alkaloid-Producing Plants

Tropane alkaloids are a specific class of alkaloids with a distinctive bicyclic tropane ring ($C_8H_{15}N$) in their chemical structure, and they occupy an important position in medicine for their rich therapeutic potential [14]. These molecules have a long and intriguing history of use by humans and scientific attention on their biosynthesis [15–19] and evolution [20–22] is well documented. The name tropane is given to the bicyclic saturated structure (N-methyl-8-azabicyclo [3.2.1] octane) denominated by Holmes as "nortropane" [23]. TAs are generally toxic molecules accumulated in many plant families, such as Convolvulaceae, Proteaceae, Rhizophoraceae, Brassicaceae, Erythroxylaceae, and in the Solanaceae plants [24]. In this review, we will focus on poisonous medicinal Solanaceae plants, i.e., with the *Datura stramonium*, *Hyoscyamus niger*, and *Atropa belladonna* (Figure 1) species being the most known source for accidental TA consumption [25].

3.2. Biosynthesis of Tropane Alkaloids

Over the past 200 years, researchers worldwide have shown much interest in the tropane alkaloids (TAs) class. Since 1830 onward, when the first chemical isolations of TAs started with atropine from *Atropa belladonna*, hyoscyamine from *Hyoscyamus niger*, and scopolamine from *Scopolia japonica*, then followed in 1860 by cocaine's isolation from *Erythroxylum coca*, these molecules have had an extensive usage in clinical medicine for their peculiar biological proprieties and distinct physiological effects in humans. The biosynthetic pathway of tropane alkaloids involves a complex coordination of independence enzymes which catalyze sequential reactions which also undergo a wide evolutionary diversification among species [26]. For example, Wang and colleagues (2023) compared the genomes of *Erythroxylum novogranatense* (Erythroxylaceae) and *Anisodus acutangulus* (Solanaceae) to understand at a genetic and structural level the evolutive changes in key enzymes

responsible for the structural differences in tropane alkaloid (TA) formation as for cocaine and hyoscyamine [21,27].

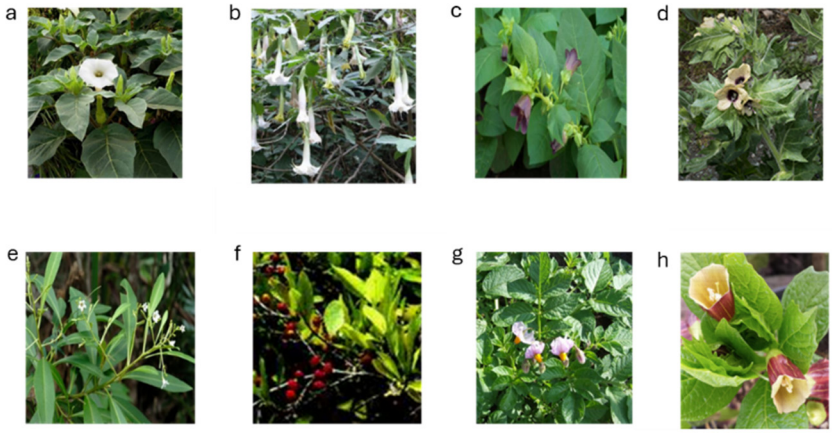

Figure 1. Tropane alkaloid (TA)-producing plants. (**a**) *Datura stramonium*, (**b**) *Brugmansia*, (**c**) *Atropa belladonna*, (**d**) *Hyoscyamus niger*, (**e**) *Duboisia* spp., (**g**) *Solanum tuberosum*, and (**h**) *Scopolia* representative species of Solanaceae family; (**f**) *Erytroxylum coca* from Erythroxylaceaea family.

The distinctive evolutionary pathway between Solanaceae and Erytroxylaceae, besides divergence in TAs molecules formation and distinct enzymatic reaction, relies also on a different biosynthetic location. So far, the biosynthesis of tropane alkaloids in Solanaceae takes place in roots and then TAs are translocated to the aerial parts, whereas in Erythroxylaceae, TA formation and storage occurs in the leaves [18,21]. Tropane alkaloids consist of more than 200 known compounds in various plants, and the biosynthesis of the tropane ring formation is a shared route. The biosynthesis of the TAs starts with the decarboxylation of the amino acids ornithine or arginine by ornithine decarboxylase (ODC, EC 4.1.1.17) or arginine decarboxylase (ADC, EC 4.1.1.19) to form the polyamine putrescine. Mono-methylation of putrescine via methyltransferase (PMT, EC 2.1.1.53) leads to N-methylputrescine. Then, oxidation of the primary amino group by diamine oxidase (DAO, EC 1.4.3.22) yields 4-(methyl-1-amino) butanal. Next, spontaneous cyclization dehydration takes place, with the formation of the reactive N-methyl-Δ-1-pyrrolinium cation which is the precursor of all TAs. This monocyclic precursor is further converted into a corresponding 4-carbon side chain β-ketoacid intermediate by the action of two acetyl coenzyme A (AcCoA) ester molecules. The oxobutanoic acid can cyclize to exo-carboxy tropinone. Then the tropinone can be the substrate to produce both/either tropine (3α-tropinol) by tropinone reductase (TR I, EC 1.1.1.206) and/or pseudotropine (3β-tropinol) by tropinone reductase (TR II, EC 1.1.1.236) [28]. Structural differences among TAs derive from the tropine esterification with a variety of acids which are opportunely classified based on the number of carbons in the tropane skeleton and stereochemical conformation. Hydroxylation at the C3 position of tropane determines two stereoisomers, tropine (3α-tropanol) and pseudotropine (3β-tropanol), depending on the orientation (α or β) of the hydroxyl group [29]. The activity of TR I leads to the most well-known TAs, hyoscyamine, atropine, and scopolamine. In detail, TR I esterified tropine with phenyllactic acid producing littorine, which in turn isomerizes to form hyosciamine. Subsequently, hyoscyamine can be transformed into scopolamine via the enzymatic activity of hyoscyamine 6β-hydroxylase (H6H) [30]. TRII leads to the formation of calystegines through pseudotropine. Calystegines are polyhydroxylated nortropane alkaloids (NTAs) whose structure consists of the single tropane ring with three to five hydroxyl groups in various positions that are divided into three principal groups (groups A, B, and C) [31]. High levels of group A and B calystegines were found in food from the Solanaceae family, such as potatoes, peppers, paprika, and eggplants. Although all TAs have a high degree of structural similarity due to their tropane ring, they

exhibit considerably different pharmacological effects [28]. Only calystegines have not been reported to have toxic effects on human and animal health, Figure 2.

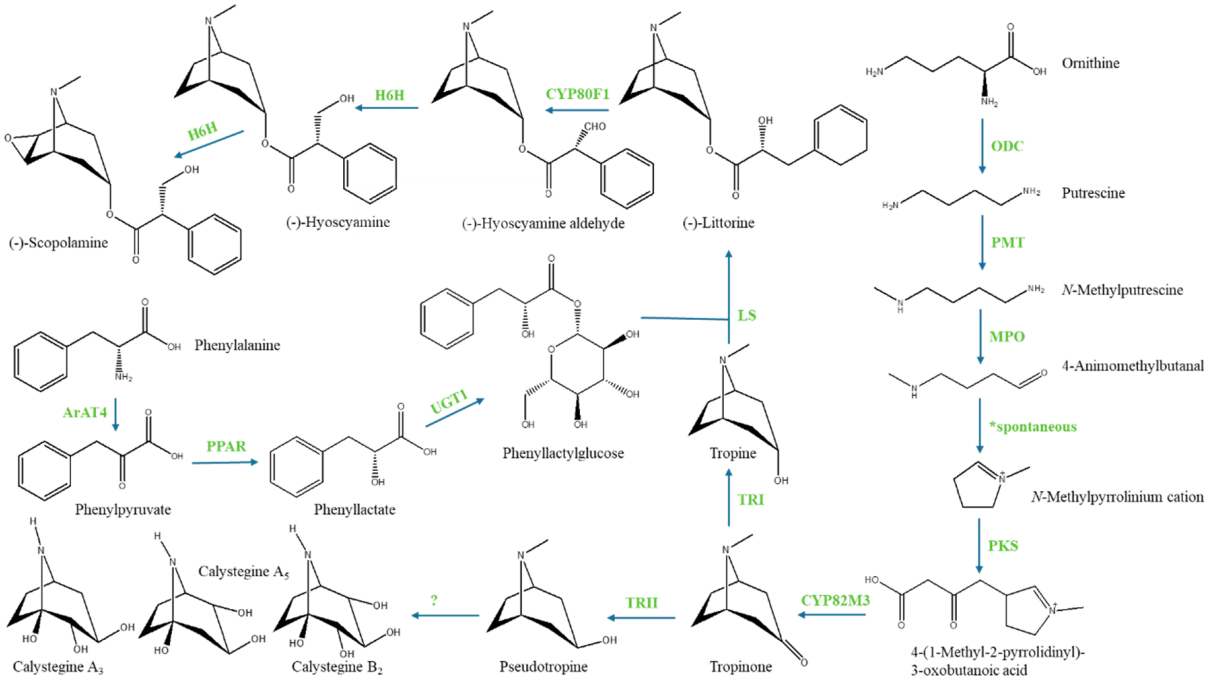

Figure 2. Biosynthetic pathway for tropane alkaloids formation. Enzyme symbols: ODC = ornitine decarboxylase; PMT = putrescine *N*-methyltransferase; MPO = *N*-methylputrescine oxidase; * = spontaneous cyclization; PKS = pyrrolidine ketide synthase; CYP82M3 = tropinone synthase; TRI = tropinine reductase I; TRII = tropinone reductase II; LS = littorine synthase; CYP80F1 = littorine mutase; H6H = hyoscyamine 6β-hydroxylase; ArAT4 = aromatic amino acid aminotransferase; PPAR = phenylpyruvic acid reductase; UGT1 = phenyllactate UDP-glycosyltransferase.

3.3. Effect on Humans and Mechanism of Action

Tropane alkaloids, along with other alkaloids, serve as a powerful defense mechanism for plants, reducing herbivore attack and inhibiting the growth of competing vegetation. However, these showed activity also against vertebrate animals representing a worrying source of contamination for food and feed [32,33]. Human and animal exposure to TAs is primarily associated with the dietary consumption of some plants of the Solanaceae family such as *S. tuberosum* L. (potato), *S. lycopersicum* L. (tomato), *S. melongena* L. (eggplant), and *Capsicum annuum* L. (pepper). Seeds, leaves, roots, fruits, and flowers are frequently responsible for cross-contamination during harvest, and their toxicity ranges from mildly irritating to fatal [34]. TAs have a long history of intentional consumption by humans. In a recent work, the use of hallucinogenic drugs was evidenced during the bronze age about 3600 years ago [35]. Atropine and scopolamine were mostly detected via the analysis of human hairs from a burial site in Spain. This indicated the capability of these molecules to cross the blood–brain barrier to affect the central nervous system [36]. It is likely that these have been used to induce delirium, hallucinations, and altered sensory perception as part of ritualistic ceremonies by shamans. Their use as psychoactive drugs has been described also during the Medieval age [37]. For example, *Datura stramonium*, *Mandragora officinarum*, *Hyoscyamus niger*, and *Atropa belladonna* have been used as sedatives, sleep inducing agents (henbane), aphrodisiacs, and panaceas (mandrake). Besides the pharmacological activities as narcotic analgesics or hallucinogens, TAs are considered model drugs for their anticholinergic effects [28]. For example, atropine, hyoscyamine, and scopolamine are primarily antagonists of the muscarinic acetylcholine (Ach) receptors and can induce several side effects based on the amount ingested [38]. Ach, acting as a neurotransmitter in many parts of the body, is most commonly associated with the neuromuscular junction. Typically,

acetylcholine is an excitatory mediator involved in numerous physiological functions, such as regulating cardiac contractions and blood pressure, intestinal peristalsis, and glandular secretion. Hence, the downstream effects of TAs have been well known since the beginning of the previous century. Inhibition of the acetylcholine receptors of the parasympathetic nervous system determines constriction of the pupil, vasodilation, and moderation of the heartbeat [39]. This is the reason TAs have been and still are widely used drugs for the treatment of motion sickness, ophthalmic surgery, and for bradycardia treatment [40,41]. TAs, for their parasympatholytic mode of action, are classified as essential medicines by the World Health Organization for the treatment of organophosphate, pesticide poisoning, motion sickness, gastrointestinal spasms, cardiac arrhythmia, Parkinson's disease, anesthesia, analgesia, cough, and asthma relief [42,43].

A dose of 10 mg or above of atropine and scopolamine is able to produce direct toxicity [38,44]. Clinical symptoms of their toxicity are change in heart rate, decreased production of salivary secretions, bronchial and sweat glands, pupil dilation, paralysis of accommodation of the eye, inhibition of micturition, reduction in gastrointestinal tone, inhibition of gastric acid secretion, hallucinations, and death [45]. Typically, symptoms of poisoning of TAs occur 30–60 min after consumption, and the toxin is excreted from the body from 12 to 48 h with no long-term health effects [46].

Unlike the tropane alkaloids described above, the function of calystegines in plants as in humans needs further research. Calystegines are responsible for the bitter taste of vegetables and their ecological role might be for herbivore deterrence. In humans, they function as glycosidase inhibitors thus having the capability to block the carbohydrate metabolism inducing possible lysosomal storage toxicity, but no toxic chronical effects have been reported so far [47]. The calystegines, owing to their hydrophilic nature and consequent inability to pass the blood–brain barrier, are unable to show psychoactive activities [28,36]. The lack of data on their toxicity in humans does not generate concerns on food security; therefore, no strategies for their reduction have been envisaged besides for increasing food palatability in calystegine-producing vegetables. Nevertheless, more investigation on the clear ecological role of this class of tropane alkaloids is needed to prospect the potential use of calystegines as natural pesticides in sustainable farming practices.

4. Biosynthesis and Health Implications of Pyrrolizidine Alkaloids

4.1. Pyrrolizidine Alkaloid-Producing Plants

Pyrrolizidine alkaloids (PAs) are a class of alkaloids based on the heterocyclic pyrrolizidine motif (C7H13N). These carcinogenic and hepatotoxic phytotoxins occur in approximately 3% of the world's flowering plants, and their role is to act as deterrents against herbivores and parasites. They are prevalent in the plant families of Asteraceae (tribes Senecioneae, genera *Senecio* and Eupatorieae), Boraginaceae (genera *Heliotropium*, *Echium*, and *Symphytum*), Apocynaceae, Orchidaceae, one genus of the Fabaceae (genus *Crotalaria*). Many plant species which accumulate PAs are used in traditional medicine for the treatment of various diseases, others are used as foods or fodder crops [48,49] as seen in Figure 3.

Food contamination by PAs occurs directly through the co-harvesting of food crops intended for humans and animals with PA-producing plants and indirectly through carryover into animal products such as honey, eggs, and milk [50]. Recently the occurrence of dehydro PAs in teas and herbal teas has gained increasing attention from the EU, due to the high levels of dehydro PAs found in commercially available teas and herbal teas in Germany and Switzerland.

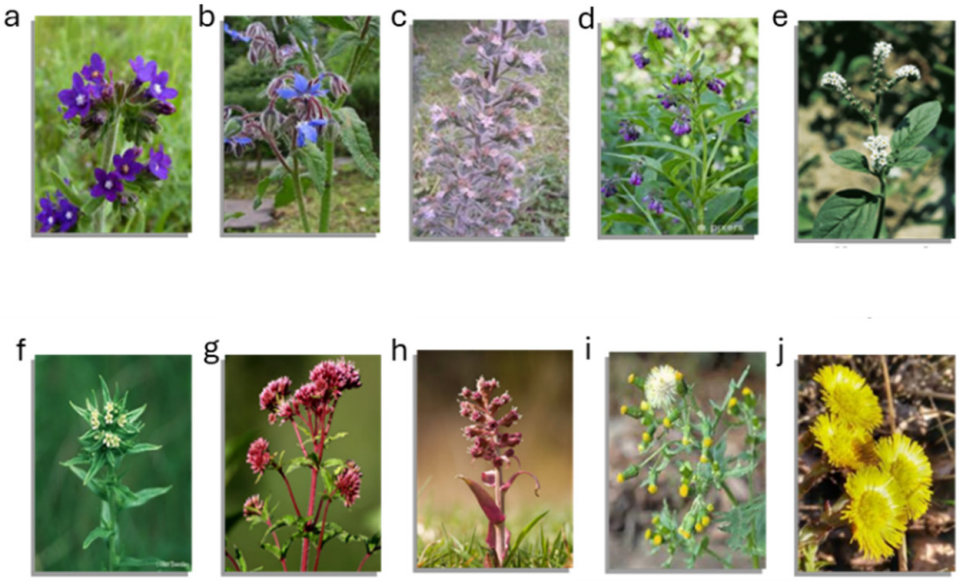

Figure 3. Pyrrolizidine alkaloid (PA)-producing plants. (**a**) *Anchusa officinalis*, (**b**) *Borago officinalis*, (**c**) *Echium italicum*, (**d**) *Symphytum officinale*, (**e**) *Heliotropium europaeum*, and (**f**) *Lithospermum officinale* representative species of Boraginaceae family; (**g**) *Eupatorium cannabinum*, (**h**) *Petasites hybridus*, (**i**) *Senecio vulgaris*, (**j**) *Tussilago farfara* from Asteraceae family.

4.2. Biosynthesis of Pyrrolizidine Alkaloids

The PA-content in plant material varies considerably, from trace amounts up to 19% based on dry weight, and this variability depends on numerous factors: genetic variation and heredity, tissue type and developmental stage of the plant, environmental conditions and extraction procedures [48]. In plant tissues, PAs are mainly detected as the corresponding N-oxides (PANOs) except for the seeds of *Crotalaria* spp. or in Orchidaceae [48]. Despite numerous warnings regarding PA contamination, currently, knowledge regarding their biosynthesis is still limited, while the intermediates were well defined via feeding experiments [51]. The first studies regarding the biosynthesis of PA date back to the 1960s with Nowacki and Byerrum [52] who conducted the first feeding experiments with radiolabeled precursor. Subsequently, these studies have been continued and deepened, and in the 1990s, the homospermidine synthase (HSS, EC 2.5.1.44) was identified as the first pathway-specific enzyme of PA biosynthesis [51] as seen in Figure 4. In several PA-producing species, HSS presents a highly different spatiotemporal gene expression, it is specific for distinctive tissues or cells in relation to the phenological stage of the plant [53]. The various chemical structures of PAs are characteristics of specific taxa, although there are some overlaps. The senecionin-type PAs (jacobine, jacoline, jaconine, retrorsine, senecionine, and seneciphylline) are typical of the genera *Senecio* and *Crotalaria*. Lycopsamine-type PAs (echimidine, lycopsamine, and vulgarine) are present in the Boraginaceaea family and Eupatorieae tribe. Heliotrine-type PAs (europine, heliotrine, and lasiocarpine) are characteristic of the genus *Heliptropium*. Monocrotaline-type PAs (6 ulvene, monocrotaline, and tricodesmine) were detected mainly in the genus *Crotalaria*, while triangularine-type PAs were detected in the Senecioneae tribe and Boraginaceae family. Phalaenopsin-type PAs are found in the Orchidaceae family [54].

The PA distribution is highly dynamic and the diversification in PA accumulation in different organs may indicate a defensive strategy against herbivores. Furthermore, PA expression is influenced by nutrient and water supply, as well as herbivore infestation [49].

Asteraceae species synthesize the PAs exclusively in the roots; for example, in *Senecio*, the senecionine N-oxide derives from the backbone structure of macrocyclic PAs of the senecionine type, which is biosynthesized in the roots and then translocated to shoots through the phloem, where it is further functionalized to produce species-specific PA patterns [55], while biosynthetic localization of PAs in in the Boraginaceae are more variable.

So far, the site of biosynthesis is species dependent; it can be is located in the entire shoot (e.g., *H. indicum*), in roots (e.g., *S. officinale*), or both roots and shoot (e.g., *C. officinal*) [55].

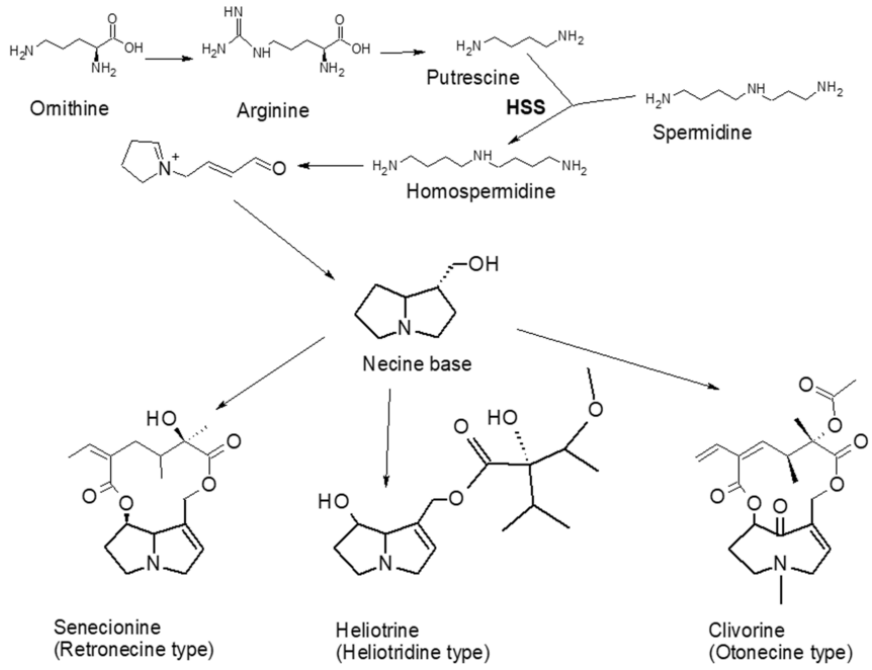

Figure 4. Biosynthetic pathway proposed for PA formation. The polyamines putrescine and spermidine are derived from the basic amino acids ornithine and arginine. Subsequently, homospermidine synthase (HSS) exchanges the 1,3-diamonopropane residue of spermidine via putrescine, which releases 1,3-diaminopropane and forms symmetric homospermidine. Oxidation of homospermidine to 4,4′-iminodibutanal initiates cyclization to pyrrolizidine-1-carbaldehyde. Desaturation and hydroxylation via unknown enzymes form retronecine, heliotridine, and otonecine type PAs.

The chemical structure of PAs consists of a bicyclic necine base to which aliphatic mono- or di-carboxylic acids, the necic acids, are generally linked via esterification. PAs can occur in plants as tertiary amines or N-oxides (PANOs), which can easily be converted back to the tertiary amines via a reduction reaction [56]. Depending on the presence or not of a double bond between C1 and C2 of the necine base, PAs can be grouped into 1,2-unsaturated PAs and saturated PAs.

1,2-unsaturated PAs are considered the most toxic because they can be oxidated in highly reactive pyrroles, which have been related to severe cases of hepatotoxicity, as a consequence of acute toxicity, and carcinogenic and genotoxic effects, as a consequence of chronic toxicity [49].

The necine bases commonly found in plants are the following: retronecine, heliotridine, otonecine, supinidine, trachelanthamidine, and platycerine (Figure 5). The first four types have an 1,2-unsaturation in the pyrrolizidine ring and are therefore considered to exhibit major toxicity whereas the platynecine and trachelanthamidine types have a saturated necine base and are generally regarded as non-toxic. Except for the otonecine type, in which N-oxides cannot be formed, N-oxides of the other types naturally occur and often coexist with basic PA forms in plant materials. On the other hand, the type of esterification between the necic acids and the necine base allows for differentiation among three further types, which are monoesters, open-chained diesters, and cyclic diesters (Figure 5). The esterification often occurs at C-7 in monoesters and at C-7 and C-9 in open-chained and cyclic diesters [48,56].

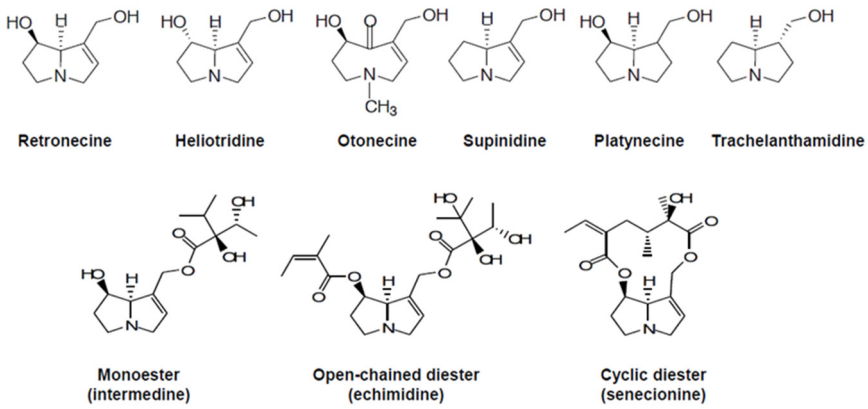

Figure 5. Necine base types and types of esterification depending on the linkage between the necic acids and the necine bases.

4.3. Effect on Humans and Mechanism of Action

PAs are considered among the most widespread natural toxins as they can affect wildlife, livestock, and humans through feed and food contamination. PA-poisoning in humans can cause acute (liver damage) and chronic (liver cirrhosis and pulmonary arterial hypertension) toxicity phenomena [57]. In 2011, the EFSA's Panel on Contaminants in the Food Chain (CONTAM) recognized the 1,2-unsaturated PAs as the most dangerous forms for human health and decided to focus on them for the risk assessment [48].

After ingestion and absorption, PAs (pyrrolizidine alkaloids) undergo metabolic activation in the liver, catalyzed by cytochrome P450 enzymes (CYP3A4 and CYP2C19). For retronecine and heliotridine-type PAs, hydroxylation at the C-3 and C-8 positions forms hydroxynecine derivatives, while otonecine-type PAs undergo oxidative N-demethylation followed by ring closure and dehydration. These reactions produce reactive dehydropyrrolizidine intermediates, leading to the formation of pyrrolizinium ions. These metabolites bind to macromolecules like glutathione (GSH), DNA, and proteins, resulting in detoxification or further reactions causing DNA adducts and oxidative damage. Although GSH conjugates might be considered detoxification metabolites, it has been reported that they also act as reactive metabolites, causing DNA adduct formation [58]. Moreover, GSH conjugation is crucial for detoxification, but depletion of GSH can lead to severe liver damage. Reactive dehydro PAs bind to liver proteins and DNA, causing genotoxicity and carcinogenicity, potentially leading to veno-occlusive disease and tumors [59].

5. Food and Feed Contaminations of TAs and PAs

There are various reports on cases of human poisoning due to contamination of food with plants containing TAs and PAs. Casado et al., 2024 reported data obtained within the last 3 year (from 2020 to 2023) from the European Rapid Alert System for Food and Feed (RASFF), highlighting the main kinds of food products frequently contaminated and their greatly incremented contamination with these alkaloids in recent years, 20 for TAs and 51 for PAs [33]. *Datura stramonium*, *Hyoscyamus niger*, and *Atropa belladonna* species can be responsible for TA (e.g., hyoscyamine, scopolamine, and atropine) cross-contamination with different food products for their similarity in morphological and botanical traits to other frequently consumed vegetables of the same family such as *Capsicum*, *Lycium*, and *Solanum.* This is particularly true for *Datura* seeds, which, resembling capsicum and cereals seeds, have been frequently mistakenly ingested. Moreover, these plants are globally distributed in all the warm regions of the world with a strong invasiveness [34,60]. The most representative example of invasive plant for TA contamination is *Datura stramonium*. This species, originating from North America, has become an invasive plant species in temperate and tropical climates worldwide. Its large-scale spread is significantly boosted by increased atmospheric CO_2 levels. The species' weedy nature is evident from its ability to thrive and produce a high seed output even with limited water availability. The seeds, resembling

buckwheat and other grains in size and shape, are major contaminants of staple food cereals. Moreover, organic farming, which avoids pesticides, further increases this risk by allowing more weed growth, potentially contaminating flours and derived products such as pasta, bread, and baby foods. There are well-documented episodes of poisoning in which *Datura* seeds have been the cause. For example, these seeds were responsible for the contamination of cereal products distributed by the World Food Programme (WFP), which led to over 300 hospitalizations and 5 deaths in 2019 [61]. In Italy, potential cross-contamination with toxic plants from the *Datura* genus caused toxicity in spinach in 2022 [62] when high levels of atropine and scopolamine, reaching 4642 µg/kg and 8158 µg/kg, respectively, were detected.

Until now, policies and regulations for TAs in food are rather insufficient. In 2013, a study on healthy young male adults exposed to TAs [63–65] allowed European Food Safety Authority (EFSA) to define an acute reference dose (ARfD) of 0.016 µg/kg for body weight, given by the sum of (−)-hyoscyamine and (−)-scopolamine. Consequently, in 2020 and 2021 the first European regulation was published to implement the monitoring of plants containing these toxins, then reported as high-risk food [66,67]. In April 2023, these regulations were edited, reworked, and expanded for relevant food products, setting the maximum levels for atropine and scopolamine. In detail, the regulation currently observed for the control of these contaminants [67] has set a limit of 5–15 µg/kg for unprocessed grains and a level of 25–50 µg/kg for dry products, such as herbals, and 0.2 µg/kg for liquid products, such as tea and herbal infusions.

The *Heliotropium* genus of Boraginaceae and the *Crotalaria* genus of Fabaceae have been reported to be the primary sources of pyrrolizidine alkaloid (PA) food poisoning in humans. Plants belonging to these genera may be present as weeds in cereal and legume crops, and their seeds can accidentally be mixed with the main crop. As a general rule, the closer is the relationship between the food and the PA-producing plant, the higher is the potential level of PAs in the final product [48]. Many plants in the Boraginaceae family are also appreciated for the quality of the honey they produce, although this can pose a risk if the honey becomes contaminated with PAs from these plants. Indeed, honey was the first food product to raise concerns about its safety regarding the contamination of PAs, both because it is a widely used product and is mostly consumed by vulnerable subjects, such as children [68]. To date, 25 PAs and 20 PANOs have been researched in commercially available honeys; among the most investigated PAs are echimidine, lycopsamine, retrorsine, senecionine, seneciphylline, heliotrine, senkirkine, intermedine, lasiocarpine, and monocrotaline, while among the PANOs, senecionine N-oxide, retrorsine N-oxide, monocrotaline N-oxide, and seneciphylline N-oxide are the most investigated ones. According to studies in the literature, the percentage of contamination of honey samples varies from 17% to 91%, and the PAs responsible for the contamination are echimidine- and lycopsamine-like compounds [69]. Celano et al. analyzed 76 honey samples, and 96% of samples were contaminated by one or more PAs. Echimidine was the most abundant PA (83% honey samples), followed by intermedine and lycopsamine detected in 69% of the analyzed samples [70,71]. Other food categories at risk of contamination by PAs are botanical preparations, such as teas, herbal infusions, and plant-based dietary supplements. A recent study of Mulder and co-workers analyzed 168 samples of teas and herbal infusions regarding the presence of 28 PAs and found that 91% of them were contaminated by one or more PAs. Senecionine, retrorsine, and their N-oxides were the most detected PAs in high concentrations, together with lycopsamine and heliotrine-like compounds [72]. Regarding plant-based dietary supplements, a study conducted by EFSA in 2016 revealed the presence of a number between 9 and 28 PAs in 278 samples of dietary supplements. The analyzed samples contained extracts of different PA-producing plants (*Borago officinalis*, *Eupatorium cannabinum*, *Symphytum officinale*, and *Tussilago farfara*); among them, lycopsamine, intermedine, and their respective N-oxides represented the PAs detected in the highest concentrations while senkirkine was found in 80–90% of the samples containing extracts of *Tussilago farfara* [73].

The European Commission selected 28 PAs as relevant in food samples [73], based on the EFSA 2011 reports [48,73–75] and considering the available analytical standards. Subsequently, the European Commission published the Regulation (EU) 2020/2040 concerning the maximum residue levels of pyrrolizidine alkaloids in certain foodstuffs [76]. The regulation sets maximum levels (μg/kg) of 35 PAs (21 PAs and 14 coeluting isomers) for 11 foodstuff categories [76]. The International Agency for Research on Cancer (IARC) evaluated several PAs and PA-containing plants and classified riddelliine, lasiocarpine, and monocrotaline in group 2B (possibly carcinogenic to humans), and retrorsine, retrorsine N-oxide, jacobine, senkirkine, and seneciphylline in group 3 (not classifiable) [77,78].

6. Molecular Markers for Detecting Invasive Plant Contaminants in Food Crops

Involuntary ingestion of poisonous plants is a frequently reported issue worldwide, and the number of species involved is increasing due to the incorrect identification of toxic and edible plants. In the food supply chain, it is becoming more necessary to develop authentication systems that achieve more accurate identification and reliable plant classification. DNA-based markers are considered particularly reliable and stable for identifying plant species that are morphologically identical [79]. In the context of food contaminants, DNA molecular markers are specific DNA sequences that identify specifically the presence of unwanted plant species or adulterants by providing a specific genetic fingerprinting. The detection of these specific DNA sequences, in order to be useful in certification labs, must easily and quickly differentiate between the contaminant and the intended food source.

In the last decade, several studies have emphasized the use of molecular markers in the authentication of plant-origin foods, focusing on species identification and discrimination [80,81]. For example, sequence-characterized amplified region (SCAR) markers, developed from the older technique of random amplified polymorphic DNA (RAPD), have been employed to detect safflower (*Carthamus tinctorius*) adulteration in saffron (*Crocus sativus* L.) [82].

Thanks to the increasing knowledge of the DNA sequences of several species, there is a greater possibility of establishing a database of invasive plant DNA barcode sequences [83]. DNA barcoding represents a highly effective method for species identification [84]. This technique uses a short section of DNA from specific genes to accurately identify species, especially when initial morphological identification provides only approximate results [85]. DNA barcoding has also been proven successful in detecting sources of phytotoxic pyrrolizidine alkaloids (PAs) and other toxic compounds. For instance, herbal material from the *Heliotropium* genus (Boraginaceae) has been detected in spice seeds using this method [86]. The most important aspects to consider when using DNA molecular markers to detect invasive plant contaminants are the efficacy in detecting low levels of contamination and the speed of the analysis. To this aim, DNA barcoding with high-resolution melting (Bar-HRM) analysis has been applied for species identification and authentication since it is a fast, reliable, less time-consuming, and inexpensive method. Bar-HRM analysis allows for the detection of single-base variants in a short region of DNA based on differences in the melting curve, i.e., the graphical representation of the relationship between temperature and the denaturation of DNA [87,88]. For example, HRM analysis is capable of detecting saffron admixture with other plant materials, including *Carthamus tinctorius* L., *Calendula officinalis* L., *Curcuma longa* L., *Zea mays* L., and *Gardenia jasminoides* [89]. Also based on HRM, Anthoons and colleagues [88] developed a method to detect a specific ITS2 for discriminating common toxic plants. Since it relies on small fragments detection, the authors showed that it could be applied to identify sources of intoxication even after digestion for forensic scopes. In particular, authors with Bar-HRM analysis tested successfully the presence of *Datura stramonium* in simulated mixtures with the edible *Amaranthus retroflexus*.

Molecular markers can even be used in food authenticity and traceability for end products that have undergone extensive and complex processing, as some DNA often remains intact after all industrial production steps thanks to its stability. Regarding efficacy

in identifying small amounts of contamination, recent advancements in technology, such as digital PCR, have significantly enhanced detection capabilities. Digital PCR offers higher sensitivity and precision compared to traditional quantitative PCR methods. It enables the quantification of low-abundance DNA targets by partitioning the sample into thousands of individual reactions, each tested for the presence of the target DNA. This technology allows the detection of minute quantities of contaminant DNA with high accuracy, making it particularly effective for identifying low levels of poisonous invasive plant contaminants. Additionally, digital PCR provides improved reliability and reproducibility, even in complex or degraded samples. Although there are no current examples in the literature specifically applying digital PCR to detect invasive plant contaminants, its potential in this field is promising and warrants further exploration.

A possible limitation related to barcoding plant contaminants is the availability of reference sequences in databases and the challenge of distinguishing between inter- and intra-specific genetic variation to accurately assign identity at the species level [85]. This is increasingly relevant with the emergence of new invasive plant species. In this context, High-Throughput DNA Sequencing (HTS) techniques, or Next-Generation Sequencing (NGS), combined with advanced data analysis tools, could potentially be used in the future as running methods for monitoring alien plants contaminants in food crops. In fact, with rapid advancements, these methods are becoming faster and more affordable, allowing a comprehensive detection of substitutions and undeclared components, including those with potentially toxic effects on consumers [90]. HTS and NGS provide a broader and more detailed genetic profile of food samples, overcoming many of the limitations associated with traditional barcoding methods.

7. Analytical Methods for Detection

The recent food safety issue regarding the presence of PA and TA-contaminated food products on the market has led to a significant increase in the number of developed analytical procedures to analyze these contaminants in various food and vegetable matrices. Over the years, the analysis of PAs and TAs has been performed with several analytical techniques, such as gas chromatography, thin layer chromatography, nuclear magnetic resonance, and capillary electrophoresis. Nowadays, chromatography coupled to mass spectrometry (LC–MS) is the most suitable analytical techniques for the determination of PAs and TAs. LC–MS has a high sensitivity and selectivity, allowing accurate determination of the analytes at very low concentrations in different food and vegetable matrices [34,91]. In recent years, high-resolution mass spectrometry (HRMS) is increasingly used in analysis of trace contaminants in food matrices as it enables simultaneous screening of target, suspect, and non-target compounds [92]. The high structural variety of PAs makes the analysis challenging; therefore, HRMS is extremely suitable for the qualitative analysis of PAs and the structural elucidation of unknown PAs [93]. For this purpose, analytical platforms for the identification and quantification of a high number of PAs at trace levels in various food and vegetable matrices have been developed [94,95]. Moreover, in the case of highly complex matrices, such as dietary supplements and botanicals, the sample preparation plays a critical role. A purification step based on solid-phase extraction (SPE) is often performed to obtain enriched PA and TA fractions. However, the affinity for PAs and TAs significantly varies depending on the type of SPE method used, which is a major limitation as it may lead to underestimation of certain PAs and TAs [34,96]. In the last decade, QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe), a type of dispersive SPE introduced in 2002 for the analysis of pesticide residues in plant matrices, was shown to be a promising methodology for the selective extraction of PAs and TAs from dietary supplements and botanical products prior to LC–MS analysis [34,91].

8. Perspectives to Mitigate Invasiveness and Food and Feed Contamination

This review focuses on alkaloids as food contaminants and explores molecular strategies to mitigate their toxic effects. Our understanding of plant invasiveness is enhanced

through the integration of omics technologies—such as transcriptomics, proteomics, metabolomics, genomics, microbiomics, and nutrigenomics—which provide valuable insights into the genetic, metabolic, and ecological processes driving the invasion of non-native species and their impact on indigenous ecosystems and agro-ecosystems.

By investigating both tropane alkaloids (TAs) and pyrrolizidine alkaloids (PAs), this review provides insights that benefit a broad spectrum of stakeholders, including agricultural scientists, farmers, policymakers, and food safety regulators. The findings lay the groundwork for developing novel biotechnological approaches that could either reduce alkaloid content in invasive species or leverage their unique properties for crop improvement. Beyond providing the fundamental knowledge needed to limit outbreaks of these invasive species, the research summarized here holds transformative potential by applying the adaptive strategies of invasive plants to enhance the resilience of cultivated crops. These approaches are particularly significant for enhancing food safety and promoting agricultural sustainability in the near future. Moreover, this review aims to inform farmers about practical strategies such as targeted mutation techniques and eco-friendly RNAi strategies, which could reduce the invasiveness of alkaloid-producing plants and, consequently, their potential to contaminate crops [97–100]. For policymakers and food safety regulators, this review underscores the importance of implementing rigorous agricultural practices and adopting precision agriculture technologies to minimize contamination risks. The most reliable approach to limit crop contamination involves adhering to strict agricultural practices. For example, several measures have been proposed to limit PA contamination: (1) accurate soil control through soil analysis and removal of any identified TA- or PA-producing plants; (2) utilizing crop rotation to interrupt the life cycle of invasive weeds, thereby reducing their presence and potential contamination; (3) conducting regular field inspections and promptly removing PA-producing weeds and other unwanted plants to minimize their growth alongside food crops; (4) employing hand harvesting techniques to avoid incorporating PA-containing plants or their parts into the harvested crop; (5) removing PA plants near apiaries or relocating apiaries to PA-free fields to prevent bees from foraging on PA-containing flowers, thus minimizing PA contamination in honey; and (6) using sensitive and reliable techniques for toxin quantification. Ultimately, training programs and awareness campaigns play a pivotal role in educating stakeholders about Good Agricultural Practices (GAPs) to reduce the risks of pyrrolizidine alkaloid (PA) contamination, thereby protecting public health. The growing global concern over environmental degradation and resource depletion has prompted the adoption of sustainable agricultural practices to reduce agricultural waste.

In addition to the aforementioned agricultural practices, precision agriculture can be a key application of Artificial Intelligence (AI) in smart farming. By utilizing sensors, drones, and satellite imagery to monitor crop health, soil conditions, and weather patterns, AI can revolutionize traditional methods, leading to intelligent and data-driven systems that improve sustainability, productivity, and resilience in the agricultural sector [101]. AI can also aid in the precise administration of water for irrigation, fertilizers, and pesticides, and can be used to detect contaminant species. The growing number of applications in precision farming, driven by recent advances in AI, demonstrates its potential. For instance, combining AI with machine learning techniques, such as satellite imagery and sensors, allows for predicting crop health, disease outbreaks, and potential threats, including toxic contaminations [102,103]. Overall, this predictive potential accelerates intervention, improves productivity, reduces labor costs, and increases overall efficiency.

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