THE THREAT OF PLANT TOXINS AND BIOTERRORISM: A REVIEW

Gian Marco Ludovici[1,2*], Daniela Arduini[1], Pasqualino Gaudio[1,2], Andrea Chierici[2,3], Guglielmo Manenti[4] & Andrea Malizia[4]

[1] International Master Courses in Protection against CBRNe Events, University of Rome Tor Vergata, Italy
[2]Department of Industrial Engineering, University of Rome Tor Vergata, Italy
[3]Department of Civil and Industrial Engineering, University of Pisa, Italy
[4]Department of Biomedicine and Prevention, University of Rome Tor Vergata, Italy

[*]Email: gianmarco.ludovici@alumni.uniroma2.eu

ABSTRACT

The intentional use of highly pathogenic microorganisms, such as bacteria, viruses or their toxins, to spread mass-scale diseases that destabilize populations (with motivations of religious or ideological belief, monetary implications, or political decisions) is defined as bioterrorism. Although the success of a bioterrorism attack is not very realistic due to technical constraints, it is not unlikely and the threat of such an attack is higher than ever before. It is now a fact that the capability to create panic has allured terrorists for the use of biological agents (BAs) to cause terror attacks. In the era of biotechnology and nanotechnology, accessibility in terms of price and availability has spread fast, with new sophisticated BAs often being produced and used. Moreover, there are some BAs that are becoming increasingly important, such as toxins produced by bacteria (e.g., Botulinum toxin, BTX), or Enterotoxyn type B, also known as Staphylococcal Enterotoxin B (SEB)) and extractions from plants. The most increasing records are with regards to the extraction / production of ricin, abrin, modeccin, viscumin and volkensin, which are the most lethal plant toxins known to humans, even in low amounts. Moreover, ricin was also developed as an aerosol biological warfare agent (BWA) by the US and its allies during World War II, but was never used. Nowadays, there are increasing records that show how easy it can be to extract plant toxins and transform them into biological weapon agents (BWAs), regardless of the scale of the group of individuals.

Keywords: *Biological warfare agent (BWAs)*; *plant toxins*; *biosecurity*; *biocrimes*; *bioterrorism.*

1. INTRODUCTION

The current concept of terrorism has as its main objective to threaten and terrorize large groups of humans, governments, armies or societies (Cenciarelli *et al.*, 2013; Barras & Greub, 2014). Concerning bioterrorism, it is assumed that this type terrorism involves the use of biological agents (BAs) by actors or groups, motivated by various reasons, such as political belief, religious convictions, etc., with the goal of achieving a specific aim (Christopher *et al.,* 1997; Mannik, 2009; Oliveira *et al.*, 2020). With regards to the latter topic, the debate has emerged in recent years regarding the transcendence of biological warfare, specifically with respect to public health, individual criminal acts, bioterrorism, wars and international laws directed towards the elimination of biological warfare agents (BWAs) (Gori & Tomar, 2020). At present, bioterrorism is one of the most intricate topics of discussion, posing unresolved problems and opening up new ethical challenges. Many scientists define acts of biological warfare as the absolute perversion of medical science (Atlas, 2002; Koch *et al.*, 2020; Oliveira *et al.*, 2020).

Besides being coherent and true in regards to the current political and scientific concerns, this affirmation of the transcendence of biological warfare and bioterrorism also has important historical significance. The reasoning behind the threat and menace to public health seems to be as ancient as humanity itself (Riedel, 2004). The historical study of biological warfare and bioterrorism is a matter that needs to be debated cautiously as it deals with a variety of factors that need to be controlled and examined in depth (Oliveira *et al.*, 2020). The lack of reliable scientific data on alleged bioterrorism attacks, particularly those that happened before the rise of modern microbiology and the conditions concerning any of the presumed attacks, in other words the availability to documentation, is susceptible to a multitude of manipulative factors such as political, scientific, as well as historical distance between older stories of attacks and their potential misunderstandings when interpreted with today's current objectives and motivations (Riedel, 2004; Thavaselvam & Vijayaraghavan, 2010; Klietmann & Ruoff, 2011; Barras & Greub, 2014; Lee & Kim, 2021). Given such a multitude of factors implied, it is difficult to differentiate natural epidemic events from alleged biological attacks (Koch *et al.*, 2020).

1.1 Biological Warfare in History

In ancient history, one of the well-known attempts to use BAs was the one that happened during the 14^{th} century medieval siege of Kaffa (Cenciarelli *et al.*, 2013; Oliveira *et al.*, 2020). In this event, the Tartars who attacked Kaffa spread dead and dying victims of the plague into the city in an attempt to further spread the disease (Christopher *et al.*, 1997; Wheelis, 2002). In another documented incident in Fort Pitt (USA), the British army deliberately spread smallpox among the native Indian population by presenting them with blankets and linens used by smallpox victims (Ranlet, 2000). There is historical evidence that shows attempts of spreading smallpox disease via infected British soldiers during the American Revolutionary War (1776-1781) and by contaminated clothing during the American Civil War (1861–1865) (Becker, 2004). The importance of BAs has become increasingly significant in the present century due to several wars and multiple threats spread. In spite of the 1972 Biological Weapons Convention, records have shown how accidental release and deliberate use have occurred, for example, of anthrax from a military testing facility in the former Soviet Union in 1979, along with the Iraqi army and its possession of BTX, anthrax and various aflatoxins (AFs) (e.g.. AFB and AFG) during the Gulf War (Black & Read, 2007; D'Amelio *et al.*, 2015). Other more recent examples have occurred in Dalles, Oregon ,US, where the Rajneeshees attempted to influence local elections by contaminating salad bars with *Salmonella typhimurium*, which is a bacteria that can cause food poisoning, and again in the US in 2001, where letters containing anthrax spores rekindled the fear of bioterrorism and biological warfare (Thavaselvam &Vijayaraghavan, 2010; D'Amelio *et al.*, 2015; Sharma *et al.*, 2016). All this makes it possible to see how research and development of these toxins and BAs is heavily widespread. The most important aspects linked to the use of BAs in a bioterrorism event are accessibility to the agents, the scientific experience for the management and large scale production of the latter together with accessibility in providing the correct equipment for its widespread diffusion (Kaufer *et al.*, 2020).

1.2 Biological Warfare Agents (BWAs)

BWAs are microorganisms, such as virus, bacteria, fungi, protozoa or toxins produced by the latter themselves, that give birth to diseases in humans, animals or plants, particularly when deliberately dispersed in an area (Jansen *et al.*, 2014; Janik *et al.*, 2019). These agents can cause large-scale mortality, disable large numbers of people in a short time and have serious adverse effects on human health. The use of BWAs can be hidden or obvious, and differ from conventional weapons due to several unique properties (Riedel, 2004; Sharma *et al.*, 2016). The effects of these agents are not instantaneous and require from a few hours to weeks before the symptoms appear in the affected population. Biological attacks only require a release of small amounts of viable agents (Ludovici *et al.*, 2015). For example, viruses are a small collection of genetic code, either DNA or RNA, surrounded by a protein coat. A virus cannot replicate alone. Viruses must infect cells and use components of the host cell to make copies of themselves. Often, they kill the host cell in the process, and cause damage to the host organism. Bacteria are mono-cellular prokaryotic organisms and possess a determined cell wall. Fungi are unicellular or multicellular organisms, eukaryotes and have no chlorophyll, unlike plants. Many fungal species are known to cause diseases in plants and humans (Cenciarelli *et al.*, 2013). Toxins are secondary metabolites produced by bacteria, fungi, algae, plants, fish and crustaceans (and also discovered in viruses), and are known to act in very low concentrations affecting cell functioning (Thavaselvam &Vijayaraghavan, 2010). Many plant species produce different and extremely lethal types of toxins and have the potential to be used as a BWA (Jansen *et al.*, 2014; Sharma *et al.*, 2016; Janik *et al.*, 2019; Kaufer *et al.*, 2020; Dass, 2021).

2. PLANT TOXINS

A large number of plants produce ribosome inactivating proteins (RIPs), which are catalytic enzymes that act intracellularly, inhibiting eukaryotic protein synthesis, thus leading to apoptosis and cell death (Endo *et al.*, 1998; Craik *et al.*, 2002; Giansanti *et al.*, 2010). RIPs have been identified in more than 60 different plant species, and have also been found in fungi, bacteria and algae. Currently, more than 50 type I RIPs and approximately 15 type II RIPs have been identified (Sha *et al.*, 2010; Yang *et al.*, 2016). Type I RIPs are the most numerous and are all synthesized as a single chain enzyme of approximately 30 kD. Contrarily, type II RIPs show two polypeptide chains: a smaller A-chain and a larger B-chain, connected by a disulfide bond. The lectin properties of the B chain (~35 kDa) enable toxin binding to cell-surface carbohydrates, while the A-chain (~30 kDa) possesses catalytic activity (de la Cruz *et al.*, 1995; Walsh *et al.*, 2013; Yang *et al.*, 2016). Lectins such as ricin, abrin, modeccin, viscumin and volkensin come under the group of toxic lectins of A-and B-chains. The enzyme component is not active until released by the native toxin (A + B) (Patočka & Středa, 2003; Bolognesi *et al.*, 2016; Polito *et al.*, 2019). Isolated A subunits are enzymatically active but do not have the ability to bind at the cellular level. Instead, isolated B subunits can bind to target cells (and even block the binding of the native toxin), but their activity is non-toxic (de la Cruz *et al.*, 1995). The initial binding of the B-chain to the glycoside residues on the glycoproteins and on the glycolipids on the cell membrane causes endocytosis of the toxin. All plant RIPs, including all type I toxins and the A-chains of type II toxins, are RNA N-glycosidases capable of hydrolyzing the nitrogen–carbon glycosidic bond of a specific adenosine located in the sarcin / ricin domain of the largest ribosomal RNA (Endo *et al.*, 1998; Yang *et al.*, 2016). However, recent evidence shows that RIPs not only deadenylate ribosomal RNA, but are also capable of removing adenine residues from DNA and several other polynucleotide substrates. Thus, it has been proposed to rename RIPs as polynucleotide-adenosine-glycosidases (PAGs) (Shakirova & Bezrukova, 2007).

The B-chain is able to bind the glycoside residues on glycoproteins and glycolipids, causing endocytosis of the toxin. Intracellular toxin transport occurs due to the endosomal system until reaching the Golgi apparatus, with this transport seemingly being regulated by the intracellular Ca^{2+} levels (Lord & Roberts, 1998). Subsequently, the association with the Golgi apparatus appears to be necessary for the consequent trafficking of the endoplasmic reticulum (ER) (Endo *et al.*, 1998). Once delivered to the ER, the disulfide isomerase protein can reduce the disulfide bridge between subunits, promoting retrograde transport of the A-chain (Lord & Roberts, 1998). Afterwards, at the cytoplasmic level, the A-chain can interact with the ribosome, which acts as a suicidal chaperone stimulating proper refolding and resumption of catalytic activity. It cleaves one specific adenosine residue (A4324) near the 3'-end of 28S ribosomal RNA. This targeted cleavage blocks elongation factors (EFs) 1 and 2 from binding, thus inhibiting protein synthesis (Lord & Roberts, 1998; Bolognesi *et al.*, 2016; Sowa-Rogozińska *et al.*, 2019). Ribosomal inactivation disrupts cell repair mechanisms and induces cell death by apoptosis. Unlike their type II counterparts, type I RIPs exhibit low toxicity because they are not able to bind and cross the cell membrane efficiently. In contrast, type I RIPs are cytotoxic to some cells such as macrophages (Polito *et al.*, 2019). Cells can absorb type I RIPs through the pinocytosis mechanism resulting in cell death. Recently, a new type of RIP, called type III RIP, has been isolated from *Hordeum vulgare*, the common barley plant (De Zaeytijd & Van Damme, 2017). This protein consists of an amino-terminal domain similar to type I RIP and is linked to an uncorrelated carboxyl-terminal domain with unknown function (Bolognesi *et al.*, 2016; De Zaeytijd & Van Damme, 2017).

As all RIPs are very similar to each other, consequently the poisoning symptoms are almost identical and the action mechanism of the toxic proteins is the same (de Virgilio *et al.*, 2010; Walsh *et al.*, 2013). Toxic effects of ricin have a latent period and take 2 to 24 hours to develop. After ingestion, the main symptoms are abdominal pain, vomiting and diarrhea, often with blood. The toxin causes intestinal bleeding and can also cause widespread nephritis as well as multiple necrosis in the liver and kidneys (Assiri, 2012; Moshiri *et al.*, 2016). In the myocardium, the myofibrils undergo degeneration. Within several days, there is severe dehydration, decrease in urine, thirst, burning throat, headache and the patient may die from hypovolemic shock. The patients' temperature decreases before death, and they often undergo characteristic shivering. Death occurs in exhaustion or cramp (Patočka & Středa, 2003; de Virgilio *et al.*, 2010; Walsh *et al.*, 2013).

When given parenterally, ricin is twice as toxic as the most dangerous snake venoms and is probably the most toxic parenteral substance in the plant kingdom. After parenteral administration, the patient may be present with fever, leukocytosis, and then falling blood pressure and temperature. The primary target organs are the kidney, liver and pancreas. Currently, from the literary data, abrin is the most toxic (Patočka & Středa, 2003). Due to the extreme toxicity of these compounds and their capacity to be used as BWAs, they are in the schedules of controlled BAs and toxins (Janik *et al*., 2019).

2.1 Ricin

Ricin toxin, discovered in 1888, is known as the first plant lectin from the seeds of the castor plant, *Ricinus communis* L. (*Euphorbiaceae*) (Winder, 2004). *R. communis* is autochthonous to the southeastern Mediterranean region, eastern Africa and India, but is now diffuse throughout temperate and subtropical regions. It has been cultivated primarily for castor oil (de la Cruz *et al.*, 1995; Worbs *et al.*, 2011). In Ancient Egypt, Europe, India and China, castor oil has been used for lighting, body ointments, and for purgative and cathartic use. As this plant is commonly found in the wild and often used as an ornamental plant, it is easily accessible. Ricin can be made from the waste material left over from the processing of castor oil (Audi *et al.*, 2005; Griffiths, 2011). Ricin at room temperature is stable, but can be inactivated by heat above 80 °C. After oil extraction and inactivation of ricin, the defatted mash and seed husks are used as animal feed and fertilizer respectively (Endo *et al.*, 1998; Polito *et al.*, 2019). In the last decade, ricin has been used for studies of cell biology mechanisms, immunology, treatment against AIDS and cancer (Yang *et al.*, 2016; Janik *et al.*, 2019). Castor seed poisoning is very common in countries where the plant is abundant, as only five seeds are needed to induce a toxic dose. Given its production costs and its multiple uses, castor seeds are currently being produced in more than 30 countries in the world with annual production of more than 1.5 million metric tons (Patel *et al.*, 2016). The accessibility and high toxicity of ricin toxin renders it as a high-risk asymmetric threat agent to national security and public health. Due to these characteristics, ricin is classified as a Category B agent by the US Centers for Disease Control and Prevention (CDC) (CDC, 2003). Agents in this category are considered as moderately easy to disseminate and able to cause low / middle mortality. Ricin is also monitored as a Schedule 1 agent under the Chemical Weapons Convention. This concern is due to the fact that during World War II, the US army tested artillery shells loaded with high doses of ricin (Cenciarelli *et al.*, 2013; Berger *et al.*, 2016).

2.2 Abrin

Abrin is derived from seeds of *Abrus precatorius* L. plant (*Fabaceae* or *Leguminosae*) that has more than 30 common names, one of which is rosary pea plant (Wellner *et al.*, 1995). This species of plant is native to Southeast Asia, and grows well in both tropical and subtropical areas of the world where it has been introduced (Patočka & Středa, 2003). It has been recorded in Ayurvedic medicine that the leaves of *Abrus precatorius* are laxative, expectorant and aphrodisiac, while the seeds are reportedly purgative, emetic, tonic, antiphlogistic, aphrodisiac and anti-ophthalmic. The easy availability of abrin toxin and its high toxicity lead to concerns that it could pose a severe threat to public health (Lin, 1994; Liu *et al.*, 2016). The mechanism of toxic action of abrin is identical to that of ricin, but the toxicity of abrin in mice is 75 times higher that of ricin (0.04 μg/kg for abrin as compared to 3μg/kg for ricin). The diagnosis, clinical features, treatment, protection and prophylaxis is also the same for both abrin and ricin intoxications (Patočka & Středa, 2006). Hence, abrin is classified as a Category B agent by the CDC and placed in the category of Biological Select Agents or Toxins by the US Department of Health and Human Services (HHS) (Janik *et al.*, 2019). It should be reported that in 2019, the terrorist group called Jamaah Ansharut Daulah (JAD) produced bombs containing abrin. The attack was promptly stopped by local law enforcement without causing casualties. Although there is currently no further data regarding the use of abrin as a BA, it represents one of the possible candidates for a bioterrorist attack (Cenciarelli *et al.*, 2013; Dass, 2021).

2.3 Modeccin

Modeccin is a lectin from the roots of *Adenia digitata,* an African succulent plant that is comparable in toxicity to ricin and acts by the same mechanism (Endo *et al.*, 1998). The plant does not seem to have any specific use (e.g., food, drugs or animal feed) and so is not available in quantities comparable to abrin or ricin (Patočka & Středa, 2006). However, the seeds do seem to be readily available. The subunits are isolated of modeccin (later referred to as modeccin 4B) and purified from the roots of *Adenia digitata* using affinity chromatography on Sepharose 4B (called Modeccin 4B). As previously described on the structure of lectins, modeccin also has a subunit A (~26 kDa), which inhibits protein synthesis and a B subunit (~ 31 kDa), which binds to cells (Patočka & Středa, 2006; Worbs *et al.*, 2011). A second form of modeccin is purified using affinity chromatography on acid-treated Sepharose 6B, with this form subsequently termed as modeccin 6B. The latter has a molecular weight indistinguishable from that of modeccin 4B, and consists of two subunits of 27 and 31 kDa, linked by a disulphide bond. As compared with modeccin 4B, modeccin 6B is slightly less toxic to animals, does not agglutinate erythrocytes, and is a more potent inhibitor of protein synthesis, giving 50% inhibition at the concentration of 0.31 mg/ml (Patočka & Středa, 2006).

2.4 Viscumin

Viscumin (Mistletoe lectin I, ML I), belonging to the RIPs family, was identified in the late 1980s as the main pharmacologically-active ingredient of mistletoe (*Viscum album*) extract and is largely responsible for its toxicity. Very similar heterodimeric toxic viscumin was isolated from a partial-parasite obtained from Indian western Himalayas (Patočka & Středa, 2006). The purified viscumin from this source shows considerable sequence and structural differences with the European viscumin. The root mean-square-deviations (rms) calculated for α–carbon atoms of European ML-1 and Indian viscumin shows higher deviations for the A chain and lower for the B chain (Endo *et al.*, 1998). The highest deviations are found for the residues on the surface. The association of A- and B-subunits is predominantly hydrophobic in nature (de la Cruz *et al.*, 1995; Endo *et al.*, 1998; Patočka & Středa, 2006; Worbs *et al.*, 2011). In terms of toxicity it is comparable with the ricin, and like all RIPs, it has the same mechanism of action previously described (Ochocka & Piotrowski, 2002).

2.5 Volkensin

Volkensin is a lectin from *Adena volkensii* (kilyambiti plant) that is comparable in toxicity to ricin and has the same mechanism of action (like a RIP) (Olsnes *et al.*, 1982; Endo *et al.*, 1998). The plant is a relatively unattractive and toxic succulent plant found in Africa that appears to be of little interest. However, it has proven useful as a research reagent in neurology because of its ability to be taken up and transported by some types of nerve (Olsnes *et al.*, 1982; Stirpe *et al.*, 1982). There may be pressure to develop commercial sources for the research community (Patočka & Středa, 2006).

3. PLANT TOXINS AS BIOWEAPONS

During World War I, ricin was taken into consideration as a potential offensive BWA (Pita, 2009; Cenciarelli *et al.*, 2013). However, the thermal instability (stable under 80 °C) of ricin constrained its initial use in exploding shells, while ethical and treaty issues limited its use as a poison or blinding agent (Moshiri *et al.*, 2016; Polito *et al.*, 2019). The war ended before ricin was weaponized and tested as BWA (Morse, 2012; Cenciarelli *et al.*, 2013). During World War II, ricin was produced, armed into W-bombs (bombs containing ricin) and tested, but apparently was never used in battlefield. Interest in ricin continued for a short period after World War II, but soon subsided when the US Army Chemical Corps began a program to weaponize another more lethal agent, sarin (Seto *et al.*, 2007; Cenciarelli *et al.*, 2013). During the Cold War, the Soviet Union also studied ricin as a possible BWA. Ken Alibek, a former top official involved in Russia's BWA program who defected to the US, claimed that Russia developed ricin toxin as BWA, and that the ricin toxin used against the Bulgarian exiles Georgi Markov and Vladimir Kostov was created in Russian laboratories (Roxas-Duncan & Smith, 2014). In 1989, around 10 L of concentrated ricin solution was produced in Iraq, some of which were used as a payload in artillery shells. In addition, further evidence demonstrated the manufacture and storage of large quantities of AFs and BTX in Iraq (Riedel, 2004). In 1992, around 120 tons of castor beans were identified through non-government sources in Iran, presumably for the production of ricin, while in 2001, ricin was found in Afghanistan after the fall of the Taliban government (Cenciarelli *et al.*, 2013). Although the potential use of ricin as a military weapon has been studied, its usefulness as a weapon of mass destruction as compared to conventional weapons still remains controversial. It has been estimated that eight tons of ricin should be aerosolized over an area of 100 km^2 to obtain about 50% of losses, while only a small quantity of kilograms of anthrax spores would have the same effect (Ludovici *et al.*, 2015). Furthermore, large-scale ricin dispersion is logistically impractical. Therefore, even if ricin is easy to produce, it is not so likely that it could cause large-scale victims as compared to other possible agents (Madsen, 2001). Abrin is not known (to date) to have been used successfully in any wars or terrorist attacks, but there has been an attempt made by a terrorist group to incorporate abrin poison into suicide bombs (Dass, 2021). However, a large number of abrin poisoning incidents have been documented (Roxas-Duncan & Smith, 2014). Other cases of intoxication were caused by exposure to modeccin, viscumin and volkensin. Many scientists, mainly from the US Department Homeland of Security (DHS), have repeatedly pointed to the dangers of these substances that could be used as BWAs (Cenciarelli *et al.*, 2013).

3.1 Umbrella Murder

Although ricin is not considered as an effective weapon of mass destruction, its potential as a BWA should be taken into account. The most emphasized case, known as the Umbrella Murder by the Bulgarian dissident Georgi Markov in 1978, is the first case in the recent history of biocrime (Papaloucas *et al.*, 2008; Musshoff & Madea, 2009; Polito *et al.*, 2019). Markov was a 49 year old Bulgarian novelist and writer who left Bulgaria to move to England in the 1970s. In London, he had published and broadcasted anti-communist points of view. On 7 September 1978, while waiting at a bus station, he felt a painful blow to his right leg and immediately saw a man with an umbrella (Polito *et al.*, 2019). The next day, he was admitted to the hospital with high fever, vomiting and difficulty speaking. He showed a wound with 6 cm of diameter with inflammation and hardening in the thigh (Musshoff & Madea, 2009). Three blood cultures were negative. His white blood cell count was 10,600 cells per µL. The next day, Markov suffered from a septic shock syndrome with vascular collapse. Subsequently, his white blood cell count rose to 26,300 cells per µL. Later, Markov stopped passing urine and the vomit became bloody. Four days after the attack, his electrocardiogram showed a complete conduction block (Papaloucas *et al.*, 2008). A few hours later, Markov died. Autopsy revealed pulmonary edema, fatty change of the liver, hemorrhagic necrosis of the small intestines, as well as interstitial hemorrhage in the testicles, pancreas and inguinal lymph nodes (Papaloucas *et al.*, 2008).

Vladimir Kostov was another Bulgarian exile who fled to Paris. On 26 August 1978, just two weeks before Markov's murder, Kostov felt a similar blow on his shoulders while on the subway. Kostov had high fever and was hospitalized for 12 days, but he recovered completely (Musshoff & Madea, 2009). X-rays showed a metallic foreign body in his back. An identical one was removed from Markov's leg (Riedel, 2004). Kostov wore heavy clothes, and perhaps that is why the metal body did not penetrate deep enough into his body to melt the wax casing. It has been estimated that the holes could have contained 500µg of lethal substance inside. Although no substance has ever been found in these two granules, agents such as BTX or SEB have been considered as possible causes (Riedel, 2004; Papaloucas *et al.*, 2008; Musshoff & Madea, 2009). The circumstances suggested that probably ricin was used in the attacks. It has always been thought that the instigator of these two murders was the Bulgarian government. Instead it was reported that ricin was produced and sent to Bulgaria by the Soviet Union. However, this has not been demonstrated (Riedel, 2004; Musshoff & Madea, 2009). Despite the KGB's denial, high-profile defectors Oleg Kalugin and Oleg Gordievsky have since confirmed the KGB's involvement (Riedel, 2004).

3.2 Ricin and Abrin: Biocrimes & Bioterrorism – 1981-2020

All major events, starting from 1981 until 2020, in which ricin was used intentionally as BW are listed as follows. An attack by terrorist organizations aimed at hitting a large number of individuals is to be considered as a bioterrorist attack; while the use, albeit intentional, carried out for example by problematic ordinary citizens or by petty criminals who could easily obtain minimal amounts of ricin is classified as biocrimes.

- In 1981, exposed CIA double agent Boris Korczak was reportedly shot with a ricin-laced pellet. He survived this assassination attempt that was probably organized by the KGB (CDC, 2003).
- In 1982, W. Chanslor, a Texas lawyer was fined and sentenced to jail for plotting to kill his wife with ricin (CDC, 2003).
- In 1985, Montgomery Todd Meeks, a high school senior, was convicted of attempted murder in a plot to kill his father using ricin (CDC, 2003).
- In 1991, members of the Minnesota Patriots Council acquired castor beans and planned to use ricin to assassinate local deputy sheriffs, US Marshals, and IRS agents. They were convicted in 1994 and 1995 under the Biological Weapons Anti-Terrorism Act (BWATA) law (Roxas-Duncan & Smith, 2014).
- On 21 April 21 1992, the Washington Post published an article about an unsuccessful attempt to poison Soviet political opponent Alexander Solzhenitsyn with ricin (Bozza *et al.*, 2015).
- In 1995, Deborah Green, a non-practicing oncologist from Kansas, US, attempted to kill her husband, Michael Farrar, a cardiologist, with ricin (Bozza *et al.*, 2015).
- In November 1999, FBI agents apprehended James Kenneth Gluck in Tampa, Florida, US for threatening to murder court officials in Jefferson County, Colorado, US with ricin (Roxas-Duncan & Smith, 2014).
- In August 2002, the Sunni militant group Ansar-al-Islam was reported to have been testing BWAs, including ricin, at a small facility in Iraq, experimenting on animals and humans (CDC, 2003).
- In December 2002, six terrorist suspects were arrested in Manchester, UK. Their apartment was serving as a ricin laboratory. Among them was a chemist who was producing the toxin (CDC, 2003).
- In January 2003, authorities arrested six Algerians in Wood Green, UK, whom they claimed were manufacturing ricin as part of a plot for a bioterrorist attack on the London Underground (Edwards & Gomis, 2011).
- In October 2003, a package and letter sealed in a ricin contaminated envelope was intercepted at a post office in Greenville (South Carolina, USA). The letter was signed "Fallen Angel" and threatened to poison water supplies if demands were not met (CDC, 2003).
- In November 2003, a letter addressed to the White House was intercepted. The letter contained a fine powdery substance that later tested positive for ricin, which investigators said was of low potency and was not considered a health risk (Bhalla & Warheit, 2004).
- In February 2004, traces of ricin were discovered on an automatic mail sorter in the mailroom of the Dirksen Senate Office building in Washington DC, which handled mails addressed to the Senate Majority Leader, Bill Frist (Bhalla & Warheit, 2004).
- In January 2005, the FBI arrested a man in Florida, USA after agents found ricin in his home (Roxas-Duncan & Smith, 2014).
- On 3 October 2006, a man from Phoenix, Arizona, US was sentenced to seven years in prison for attempting to manufacture ricin (Roxas-Duncan & Smith, 2014).
- In 2007, traces of ricin had been found at Limerick Prison, Ireland. The ricin was smuggled into Ireland from the US. in a contact lens case, to be used in a murder plot (Roxas-Duncan & Smith, 2014).
- In February 2008, authorities recovered castor beans, a weapons cache, a copy of "The Anarchist Cookbook" with a page about ricin marked, and 4 g of ricin in Las Vegas, Nevada, US (Schieltz *et al.*, 2011; Shea & Gottron, 2013).
- In June 2009, Ian Davison was arrested after the discovery of ricin at a house in County Durham, UK. Davidson, a British white supremacist and neo-Nazi, was sentenced to 10 years of prison in May 2010 for preparing acts of bioterrorism (Roxas-Duncan & Smith, 2014).

- In January 2011, the FBI arrested a man from Coventry Township, Ohio, US for unlawful possession of ricin (Roxas-Duncan & Smith, 2014).
- In June 2011, Michael Crooker was sentenced to 15 years in prison for illegally possessing ricin and threatening a prosecutor (Roxas-Duncan & Smith, 2014).
- In June 2011, a British citizen, Asim Kauser, was brought to court on charges including possessing instructions for producing ricin (Roxas-Duncan & Smith, 2014).
- In August 2011, the US government discovered information that terrorist groups were attempting to obtain large amounts of castor beans for weaponizing ricin (Shea & Gottron, 2013).
- On 1 November 2011, four elderly men from Georgia, US were arrested relating to plans to obtain ricin for use in attacks against other US citizens, as well as government personnel and officials (Bjelopera, 2017).
- On 16 April 2013 an envelope addressed to Senator Roger Wicker of Mississippi tested positive for ricin at the US Capitol's off-site mail facility. A few days later, the Secret Service announced that a letter addressed to the US President, Barrack Obama, containing a "suspicious substance", was intercepted at the White House's off-site mail facility on that day. Later that the day, the FBI confirmed that the letter tested positive for ricin (Gibb & Kes, 2013).
- In March 2014, at a student at Georgetown University, US, Daniel Harry Milzman was arrested and charged for possessing a biological toxin after admitting he made ricin in his dorm room. Milzman was later sentenced to one year in prison (Mickolus, 2016).
- On 4 June 2014, Jeff Boyd Levenderis was convicted of possessing ricin for use as a weapon and also possessing of ricin and making false statements to agents of the FBI. He was later sentenced to six years in prison (Burke, 2017).
- On 30 November 2017, 71 year old Betty Miller was arrested and charged with unregistered possession of a select agent (Burke, 2017).
- In June-July, 2018, a Tunisian man was arrested in the German city of Cologne for allegedly obtaining ingredients for the production of ricin on the internet. Furthermore, the man bought 1,000 castor oil seeds and an electric coffee grinder from an internet mail order company. A month later, the Tunisian man wife's was also arrested for complicity. Both were accused of being supporters of the Islamic State (Flade, 2018).
- In October 2018, authorities arrested a US Navy veteran in Utah over suspicious envelopes sent to the US President, Donald Trump and top military chiefs. Officials suspected the envelopes to have contained ricin (Yang *et al.*, 2021).
- In October 2019, the Indonesian police foiled a bomb attack plot by the Islamic State-affiliated terrorist group JAD, who manufactured bombs that after several analyses showed they contained abrin (Dass, 2021).
- In September 2020, a package containing ricin, which was addressed to President Donald Trump, was intercepted by law enforcement. A Canadian woman suspected of sending it was arrested when she tried to cross to the Canadian-US border (Yang *et al.*, 2021).

4. CONCLUSION

Biowarfare and bioterrorism represent a serious threat to the health of human beings and to the socioeconomic stability of counties. Among the possible agents that can be used for biothreat purposes, plant toxins represent a novel and still not completely explored field. Specifically, plant proteins with toxic effects, such as ricin, abrin, modeccin, viscumin and volkensin, have aroused interest due to their ease of availability and dangerousness for the purpose of being used as BWAs. The role of these enzymatic proteins, namely RIPs in plant physiology is not entirely clear. Based on their varied activity at the ribosomal level, different possible roles have been proposed, including antiviral activity, antifungal activity, defense against herbivores, a role in stopping cellular metabolism during periods of senescence and finally as reserve proteins.

Ricin and abrin, among others, have been used in the recent past in several occasions to perpetrate biocrimes due to their availability and ease of extraction from the plants' seeds, as well as due to their toxicity. In many cases, their use resulted in serious consequences and even in the death of the victims. The use of biological agents with the aim to perpetrate crimes is an actual and serious threat. Currently, the use of plant toxins for offensive purposes represents a real possibility, and research and development of strategies and approaches to mitigate their possible development and use cannot be neglected.

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