Blowflies & Nicotine: an Entomotoxicology Study
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Introduction

“Entomotoxicology” is the term used to describe the science involving the combination of entomology and toxicology. Entomotoxicology examines the adverse effects of chemicals on insects feeding on the remains of humans and other animals [1]. Toxicological substances ("drugs") present in remains can also accumulate in necrophagous insects. Many of these drugs affect insects, altering their rates of development and death [2]. In a forensic context, the identification of drugs in necrophagous insects may help determine the cause of death because in decomposed tissues the common toxicological analyses generally provide poor sensitivity and may yield erroneous results [3,4].

At present, only a modest number of substances and insect species/life instars have been studied and many early studies utilized analytical procedures which are now obsolete with little or no validation [5]. This is the first entomotoxicology study concerning the detection, analytical quantification and the effect of nicotine on any necrophagous entomofauna.

Nicotine is a volatile and water-soluble alkaloid present in the leaves and stems of the plants of Nicotiana species (Solanales: Solanaceae), which includes N. tabacum L., the tobacco plant [6]. The tobacco plant was considered to have therapeutic properties able to treat a wide range of disorders. However, several cases of patients treated with tobacco showed fatal or poisonous effects [7]. Nowadays tobacco is less used in medicine, but nicotine can be found in tobacco products, such as cigarettes, cigars, pipe and chewing tobacco, and refill solutions for electronic cigarettes. Furthermore, nicotine is present in various formulations of nicotine replacement therapy, such as nicotine transdermal patches, nasal sprays, inhalators, gums, sublingual tablets and lozenges [8]. In some countries, nicotine is also present in toothpastes to whiten teeth [9]. Finally, nicotine is also used as a synergist in insecticides [10].

Nicotine has acute toxicity; it is considered one of the most deadly poisons known to man and, at the same time, it’s extremely easy to come into contact with during normal daily life (e.g. buying smoking products) [11]. Nicotine can be readily absorbed across the epithelium of the lung, the nose, and through the skin and mucous, regardless of the mode of administration [11]. Therefore, there is a potential for poisoning from ingestion, injection, inhalation, skin and rectal absorption of nicotine from nicotine-containing products, including insecticides and tobacco products [12]. The literature reports a number of accidental/sudden, suicidal and homicidal cases whereby nicotine (alone or mixed with other drugs) was used [12].

Nicotine and its metabolites (e.g. cotinine), the major metabolite of nicotine can accumulate in human hair and nails [13]. In a nicotine overdose situation, the toxicological examinations will be focused on the presence of nicotine in the liver, while nicotine metabolites would provide only accessory information [13]. This research describes the development and validation of a suitable analytical method, based on GC-MS, to detect nicotine in larvae, pupae, empty puparia and adults of blowfly Calliphora vomitoria (Diptera: Calliphoridae). Furthermore, the effects on the blowfly’s survival and growth rate were examined when reared on substrates spiked with three concentrations of nicotine, sufficient to cause death in humans [14].

Experimental Design

C. vomitoria were reared in the insectary of the Entomological Lab at University of Turin from already established and maintained colonies. Adult flies were fed daily with water and sugar cubes ad libitum. Five days after emergence flies were provided with fresh beef liver to allow the ovaries to develop. After 10 days fresh beef liver was placed in the cages on water moistened paper on small plastic trays in order to allow females to oviposit.

Fly eggs were then moved on beef liver spiked with different amounts of nicotine (T1=2 ng/mg, T2=4 ng/mg, T3=6 ng/mg). Another liver was used as control. The appropriate nicotine spiking concentrations were selected based on the concentrations that would most likely cause death in a human [14].

Two samples, consisting of 30 individuals and another amounting to 1 g from each treatment were collected when C. vomitoria reached the second (L2), third (L3), post-feeding (PF) pupal (P) and adult (A) instars. Empty puparia (EP) were also collected. Each sample of 30 individuals was used for morphological analyses. Each sample weighing 1 g from each of the instars was analysed to detect nicotine.

The validation of the method for GC-MS for nicotine detection was performed according to ISO/IEC 17025 requirements and ICH guidelines [15]. The validation protocol included the quantitative determination of nicotine in larvae, P and EP: specificity, linearity, limit of detection (LOD), limit of quantification (LOQ), extraction recovery, repeatability and carry over were determined (Table 3).

Nicotine and cotinine concentrations in larvae and pupae of different treatments were analysed by one-way ANOVA and Pearson’s Chi-squared test. The level of significance was set at P < 0.05. Calculations were performed using IBM SPSS Statistics 22 statistical package.

Results & Conclusions

1. GC-MS method can detect both nicotine and its metabolite cotinine in C. vomitoria immatures (Table 1);

2. The presence of nicotine at the 3 scheduled concentrations in the food substrate did not modify the developmental time of C. vomitoria (Table 2);

3. During the pupation period larvae exposed to nicotine died dependent on the concentration of nicotine in the substrate (Table 2);

4. The resultant lengths of larvae and pupae exposed to 4 ng/mg and 6 ng/mg concentrations of nicotine were significantly shorter than the control (Fig. 2).