

# The Effects of Chemicals Used For Suicide on Insect Succession, Diversity and Development: An Animal Model

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## Abstract

It has proven that the presence of different chemicals can affect the succession patterns of necrophagous insects. A comprehensive study was designed on the effects of nortriptyline, diazinon, and aluminum phosphate on arthropod's succession and diversity on cadavers.

Sixteen rabbits in two groups were used as a model in this study, 12 of them were treated with the drugs and were placed in two habitats (sun and shade) based on the study design. For each group, one cage was considered as control. Insects were collected twice a day from the cadavers and identified.

In total, 549 necrophagous insects from five families, were collected from all the carcasses. *Chrysomya albiceps* was the dominant insect on all cadavers, except for one treated with diazinon, in which it was displaced by *Dermestes frischii*. The highest number of insects were collected on the sixth day for all cadavers. The majority were collected from the shaded cages.

Generally, the species diversity was higher for all the cadavers treated with the drugs compared to the control cadavers. The results showed that the presence of diazinon in the cadaver can repel necrophagous insects, on the contrary nortriptyline seems to attract more species/specimen.

**Keywords:** forensic entomology, arthropods succession, animal model, diazinon, aluminum phosphate, nortriptyline

## Introduction

Cadavers attract and can be colonized by different developmental stages of arthropods, most notable of them being flies (Diptera), beetles (Coleoptera), mites and isopods (Isopoda). This creates a plethora of

opportunities for utilization of forensic entomology, the study of insects and other arthropods of legal importance<sup>1</sup>. Forensic entomology gives us numerous methods to determine the time, place and in some rare cases the cause of death. They are two primary approaches for determination of minimum Post Mortem Interval (PMI<sub>min</sub>), development based and succession based<sup>2</sup>.

Aluminum phosphide (AIP), a solid fumigant which has been in extensive use since the 1940s, due to having properties such as, high toxicity and potency, insignificant impact on seed viability and not leaving

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behind considerable toxic residues<sup>3</sup>. Unfortunately, due to relatively easy access and lack of a proper antidote, its widespread use has been accompanied with an astounding rise in alphas poisoning, whether intentional or accidental<sup>4,5</sup>.

Nortriptyline, sharing the same class of pharmaceuticals with amitriptyline, is a tricyclic antidepressant occasionally prescribed in neuropathic pain treatment, which is recommended in European, UK, and USA guidelines<sup>6</sup>. Tricyclic antidepressants are usually lethal in high doses (causing cardiovascular and neurological toxicity) and have been frequently used as a method of suicide and their cure demands considerable therapeutic effort<sup>7-9</sup>.

Diazinon (O,O-diethyl-O-[2-isopropyl-4methyl-6-pyrimidinyl]phosphorothioate) is a contact organophosphate (OP) insecticide, with numerous applications in agriculture and horticulture. Several countries have been using ectoparasiticide formulations of diazinon for sheep and cattle, and in collars used for controlling parasites in pets. There have been multiple concerns about the diazinon's effects on human health, which led to a restriction of its uses in the USA, but diazinon is still commonly used across the globe<sup>10</sup>.

Several studies have been conducted regarding the effect of chemicals present in carcasses on the growth, development, survival of insect larvae and the accumulation in their bodies, which can be immensely useful in determination of PMI<sup>11-14</sup>. Based on the report (Unpublished data) of Iranian national legal medicine research center, organophosphorus pesticides (Diazinon is the most widely used phosphorus insecticide in the region) and some therapeutic drugs for patients (Nortriptyline with most local cases) and Phostoxin tablets (Which are very cheap, effective, and affordable) are the most common chemicals used for suicide in Iran.

In Iran, some studies have also been carried out on the fauna of forensically important insects on corpses (on indoor and outdoor human/animal

corpses) in different parts of the country and different geographical conditions<sup>15-17</sup>. In this study for the first time, we have designed a study on the effects of the three commonly used chemicals in suicide on arthropod's succession, diversity and development in an animal model in Iran.

## Materials and Methods

### Study area

Shiraz is the fifth-most-populous city of Iran and the capital of Fars Province. Shiraz is located in the south of Iran and the northwest of Kerman Province. It is built in a green plain at the foot of the Zagros Mountains 1,500 meters above sea level. Shiraz's climate has distinct seasons, and is overall classed as a cold semi-arid climate, though it is only a little short of a hot semi-arid climate or a hot-summer Mediterranean climate. Summers are hot, with a July average high of 38.8 °C. Winters are cool, with average low temperatures below freezing in December and January. Around 300 mm of rain falls each year, almost entirely in the winter months.

This study was done in a garden behind the Shiraz school of health. Two less crowded places were chosen for the placement of cages, one under direct sunlight and the other in shade (29.593033°N, 52.560528°E; 29.592813°N, 52.561107°E).

### Chemicals

In order to simulate the suicide methods, different chemicals including diazinon (technical 98% was purchased from Ariashimi Company), aluminum phosphate (Phostoxin® 56% TB manufactured by QCC), nortriptyline (as hydrochloride 25 mg Tablets manufactured by Abidi Company) were purchased and used in this study.

### Rabbit cages

To prevent interference by scavenger animals, the corpses were placed inside eight custom made steel cages measuring 165 by 160 by 155 cm<sup>3</sup>. Each

cage contained two corpses that had been killed by a specific chemical (in treatment groups) or method (in the control group). Four cages were placed under direct sunlight in an environment lacking dense vegetation while the other four were placed in shade. In the present study, we hypothesized that the presence of the aforementioned chemicals, exposure to sunlight and location of the rabbit carcasses would affect insect succession and diversity in two different habitats.

### Study design

This study was carried out between June and August 2019. The animal models used in this study were healthy inbred female rabbits (purchased locally from animal house) with the same age, and with an average weight of  $650 \pm 10$  g. Rabbits were chosen for this study because of their suitable size and also as pigs are not available in Iran due to religious restrictions.

We used a total of sixteen rabbits in two groups, the first group (4 cages) under direct sunlight and the second (other 4 cages) in the shade. For each group, one cage was considered as control (this rabbit euthanized by applying blunt force trauma to head, care was taken to minimize external injury)<sup>18</sup> and three cages consisted of individuals were killed using prepared chemicals and considered as treatments. One milliliter (ml) of prepared solution of diazinon 60%, one aluminum phosphate tablet, and 30 nortriptyline tablets dissolved in 10 ml water were used orally per rabbit to kill animal models in the treatment groups.

Each cadaver was placed in a corner of the cage and spaced 1.8 meters apart. Subsequently the corpses were monitored daily (twice a day) for a period of two weeks. Fly larvae were collected and half were killed with almost boiling water and then preserved in 80% ethanol; they were identified using Szpila key<sup>19</sup>. The other half were reared till maturity, and the adult flies were then identified based on their morphological characteristics using valid identification keys. Adult coleopterans found on corpses were sampled and after that identified using the following identification

keys<sup>20, 21</sup>. The other arthropods like ants that were not of forensic importance were ignored. Relative humidity and temperature of the sampling location were measured and recorded daily.

The study was conducted in accordance with the Basic & Clinical Pharmacology & Toxicology policy for experimental and clinical studies [22].

### Statistical Analysis

All statistical analyses were performed using SPSS software, version 21. The description of the collected insects in terms of order, family, genus, species, abundance and time of collecting, descriptive statistics are provided. Differences between treatments and control were tested for statistical significance using the chi-square (or Fisher exact test) for qualitative variables, and the Student's t-test (or Wilcoxon test) for quantitative variables.

### Results

In total, 549 necrophagous/predatory insects from five families (419 Calliphoridae, 90 Dermestidae, 34 Histeridae, 5 Cleridae, and 1 Staphylindae), were collected from all the carcasses. The majority collected insects (459≈84%) were from the shaded cages and 16% of samples were collected from sunlight cages. More than half of the specimen were collected during night (52.3%). Of the total collected samples, 76.3 % belonged to the Diptera order and 23.7% were Coleopterans. Overall, 305 (55.6%) larvae and 244 (44.4%) adults were collected during this study.

Overall, 10 different species were collected from all carcasses. *Chrysomia alibiceps* with 68.1% frequency was the dominant species and *Creophilus maxillosus* (0.2%) was the least collected species (Table 1). Among all groups, number of collected insects in the nortriptyline-treated group was the highest (33.5%) and in the diazinon group this amount was the lowest (12.8%). In total, the largest number of specimens were collected on day 6, which is considered as the peak of necrophilic insects' activity on the tested carcasses in the geographical conditions

of the study site.

The effect of different chemical compounds tested on the carcasses showed that, the abundance of collected arthropods in control group had a significant difference with the diazinon (P value= 0.00) and nortriptyline (P value= 0.023) treated groups, but there was no significant difference (P value= 0.78) in the frequency of collected insects between the control and aluminum phosphate groups. Number of collected insects according to kind of treatment, time of sampling (day and night), species composition, and location of the corpses (in the sunlight and shade) are shown in figures 5-8.

More species were sampled from the shaded cadavers (Figure 1), except for *Lucilia sericata*, *Lucilia ampulacea*, and *Necrobia rufipes* that were often collected from the carcasses placed under direct sunlight. Most flies were collected during the day, while a considerable increase in the number of collected beetles was observed during night.

The aluminum phosphate-treated cadavers had the highest diversity among all carcasses with nine collected species (30%) followed by diazinon and nortriptyline-treated cadavers (8, 26.5%), but only five species (17%) were sampled from the control cadavers.

Percentage of all collected specimens from the two common necrophagous orders from each corpse is presented in Figure 2. The trend of the number of collected insects (specimen) in each of the treated groups based on days after treatment is shown in Figure 3. Also, number of collected samples in each of the treated groups by their families are shown in

Figure 4.

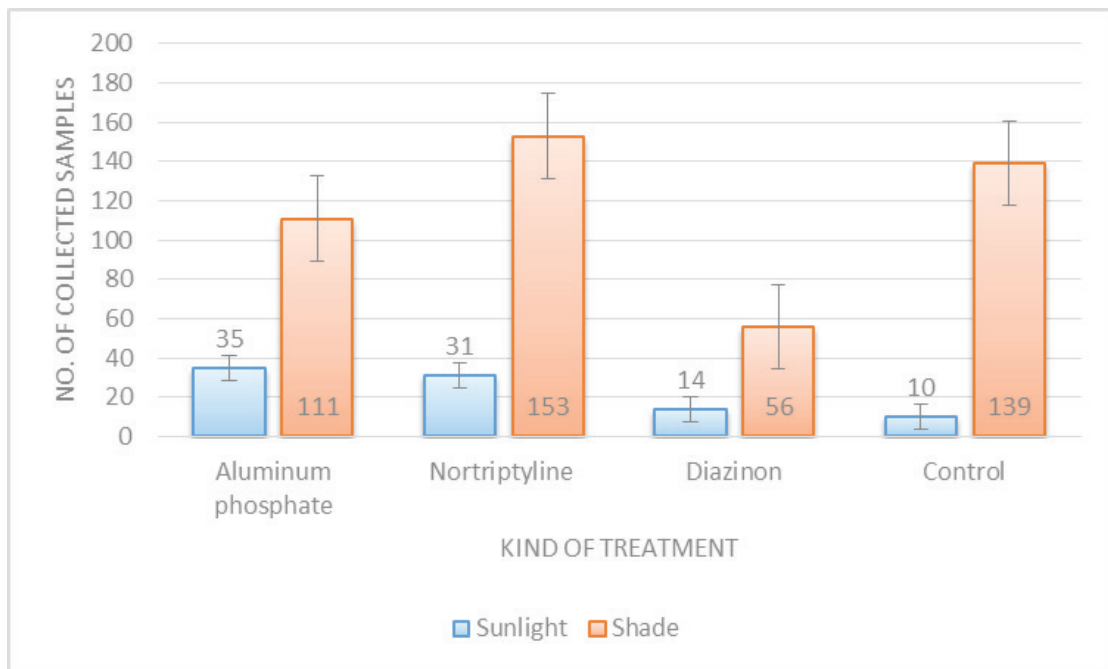
On the first day blow flies were observed ovipositing near the mouth and eyes of rabbit cadavers, no fly larvae or adult beetles were collected on this day. Only two species of forensically important insects were collected on day 2, *Dermestes frischii* (Order Coleoptera, family Dermestidae) and *Chrysomya albiceps* (Order Diptera, family Caliphoridae). *Chrysomya albiceps* were collected from the diazinon-treated cadavers, on day 2, making them the first observed Diptera, while *Dermestes frischii*, the first observed Coleoptera, were collected from the aluminum phosphate-treated corpses on the same day; no insects of forensic importance were collected from the nortriptyline-treated and control corpses on this day.

*Dermestes frischii* was the dominant species on the diazinon-treated group, while *Chrysomya albiceps* was the most abundant insect in other treatment groups (Table 1). *Creophilus maxilosus* (Order Coleoptera, family Staphlinidae) was only collected from the diazinon corpses. No insects belonging to the Cleridae family were collected from the control group. *Lucilia silvarum* (Order Diptera, family Calliphoridae) was only collected from the aluminum phosphate-treated corpses. No *Lucilia ampulacea* was collected from the control corpses. The highest number of insects were collected on day 6 on all but the aluminum phosphate-treated corpse, making it our collection peak, after which a sharp decline was observed (Figure 3).

**Appendices:**

**Table 1. Abundance of all collected necrophagous species from each corpse in different treatments groups**

Type of treatment	Kind of species										Total	Percentage (%)
	<i>L. sericata</i>	<i>C. albiceps</i>	<i>L. illustis</i>	<i>L. ampulacea</i>	<i>L. silvarum</i>	<i>D. frischii</i>	<i>C. maxillosus</i>	Hister spp.	<i>N. rufipes</i>	<i>E. strobelli</i>		
Diazinon	0	17	4	2	0	32	1	4	1	9	70	12.75
Nortriptyline	3	129	3	5	0	33	0	1	3	7	184	33.51
Aluminum phosphate	9	97	6	7	3	18	0	1	1	4	146	26.59
Control	0	131	3	0	0	7	0	1	0	7	149	27.14
Total	12	374	16	14	3	90	1	7	5	27	549	
Percentage (%)	2.18	68.12	2.91	2.55	0.54	16.39	0.18	1.27	0.91	4.91		



**Figure 1. Number of collected arthropods according to kind of treatment and location of the corpse (in the sunlight and shade)**

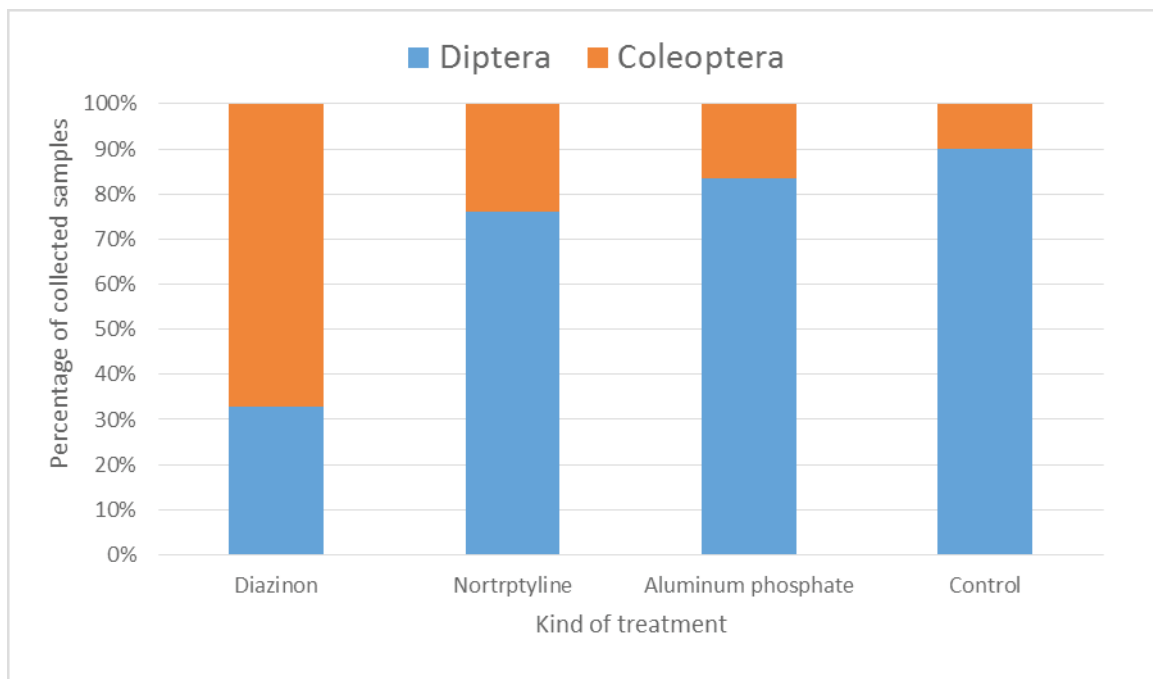


Figure 2. Percentage of collected insect orders in different treatment groups

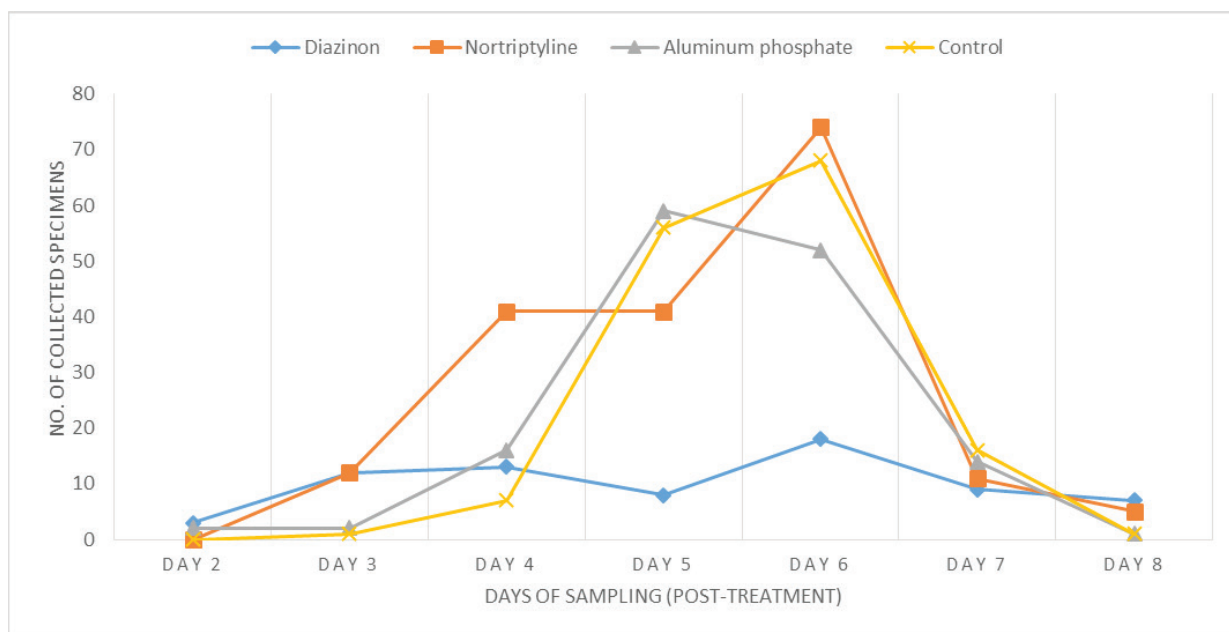
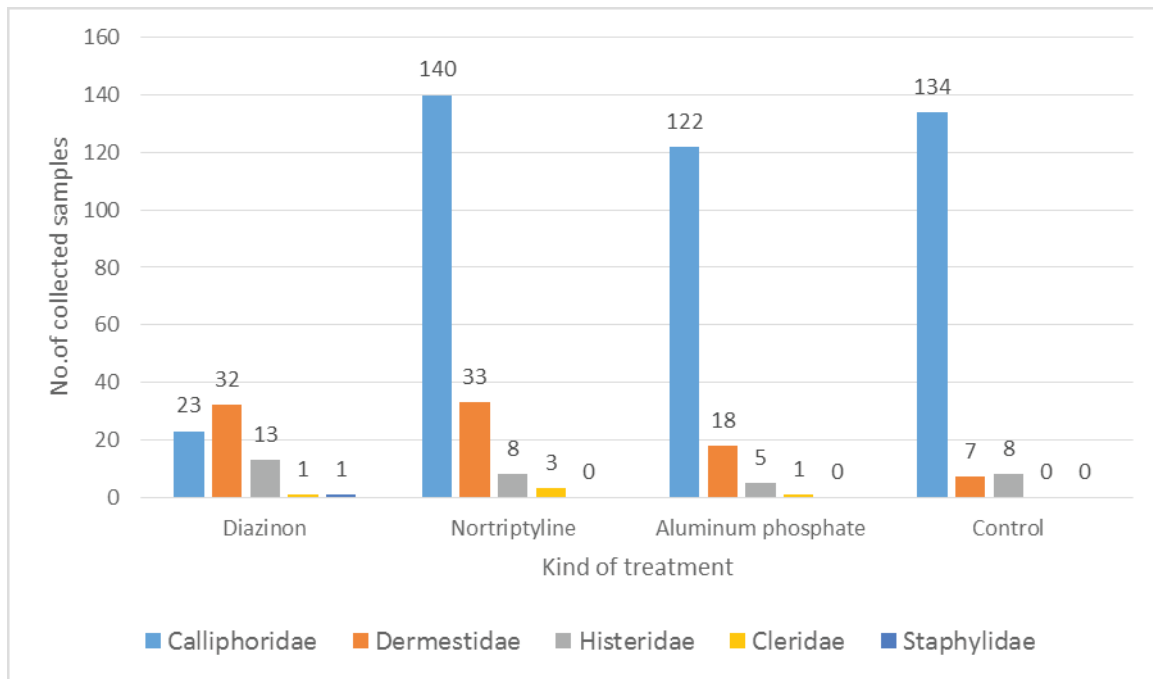


Figure 3. Number of collected insects from all tested groups according to post-treatment sampling date



**Figure 4. Number of collected samples in each of the treated groups by their families**

## Discussion

Previous research has demonstrated that the oviposition site selection done by female blowflies can be influenced by the presence of chemicals such as albuminous protein, microbial products associated with larval feeding<sup>23</sup> and several other compounds released throughout the stages of corpse decomposition<sup>24</sup>. Furthermore, bait aging and the level of available nutrients have been proven to be key factors affecting colonization pattern<sup>25</sup>.

In this study entomological data regarding the effects of 3 chemicals, which have been associated with a number of self-inflicted or accidental poisonings, on insect succession, diversity, and development was collected. 10 insect species belonging to 5 families were sampled. In our study, the collection peak between aluminum phosphate-treated corpses and other cadavers was different, which suggests the effect of this chemical on insects' activity peak as well as their succession pattern. Furthermore, the most diverse range of *lucilia spp.* were collected from the aluminum phosphate-treated corpses. The results

showed that the presence of aluminum phosphate inside the carcass can hasten the insect activity peak and shorten their life cycle. No significant effect was observed on insect development by the other tested chemicals in this study.

The results of this study showed that, in general, diazinon poisoning has a repulsive effect and reduces the tendency of necrophagous insects to settle in the affected corpses. As a slight increase was detected in the number of the collected insects from the nortriptyline corpse, it is possible that this chemical or its metabolites can attract necrophagous insects and increased their abundance.

We observed a considerable difference between the number and diversity of the collected insects in the two sampling times (day and night). The majority of the coleopteran species were collected during the night, possibly due to their nocturnal behavior. During the day, non-treated corpses were visited by a high number of flies, similar to a study done by Singh<sup>26</sup>, while other treated cadavers experienced different patterns.

Based on our results, in the control group, the frequency of collected species during day was higher than that of night. While, in treatment groups, the results were completely the opposite. These results reinforce the idea that the type of chemical present in the carcass can influence the behavior (diurnal and nocturnal activity) of necrophagous arthropods. Our results indicated that *Lucilia illustris* and *Chrysomya albiceps* were diurnal. But *Lucilia sericata*, *Dermestes frischii*, and *Euspilotus strobili* were reported as nocturnal species. Other species showed equal diurnal and nocturnal activities. Based on the results, we can conclude that the type of toxicants can greatly affect the rate of insect activity during the day or night.

As *Chrysomya albiceps* managed to be the dominant species on all but the diazinon corpse, like results from a study done by Salimi<sup>27</sup>, it is possible that the ingested diazinon had a repellent effect on this species, similar to an effect previously achieved when the outer surface of the carcasses was sprayed by the aforementioned chemical.<sup>28</sup> or perhaps attracted *Dermestes frischii*, a possible predator. As *Lucilia ampullacea* was collected from all but the control cadavers, it is possible that the addition of these chemicals acted as an attractant or hindered the rival species.

In all treated and control cadavers except the diazinon-treated group, the proportion of collected Diptera species was higher than that of Coleoptera (Figure 2). We can probably attribute this phenomenon to the possible attractant effect of diazinon on necrophagous coleopterans or perhaps other added chemicals increased the corpse's suitability as a possible oviposition site for Calliphorids.

Our results indicated that the type of chemical used to commit suicide influenced insect colonization. Diazinon attracted *Hister spp.*, *Euspilotus strobili*, and *Creophilus maxillosus* species. Whereas, nortriptyline attracted only *Chrysomya albiceps*. Also, aluminum phosphate attracted *Lucilia sericata*, *Lucilia illustris*, *Lucilia silvarum*, and *Lucilia ampullacea*. Also,

*Creophilus maxillosus*, and *Lucilia silvarum* are specific species respectively associated with diazinon and aluminum phosphate compounds. Another point to consider is that *Dermestes frischii* tends to be present in carcasses that are infused with various chemicals.

All corpses placed in the shaded cage experienced an increase in the number of collected Diptera and Coleoptera, compared to the corpses in the sunlight cage, except the nortriptyline and aluminum phosphate-treated corpses, in which the sunlight cage managed to attract more coleopterans. The diazinon and control corpses placed in the shaded cage showed lower diversity than their counterparts which were exposed to sunlight, but this was reversed in the aluminum phosphate and nortriptyline-treated corpses, similar to the results of a study done by Goff<sup>29</sup>. Generally, 84 % of the samples were collected from shaded cages. This indicates that species abundance in shaded carcasses is 4 times higher than those placed in sunlight. This result indicates that carcass placement in roofed and shaded areas will increase the abundance of insects on cadavers under geographical conditions tested in this study.

The very first colonizers of the aluminum phosphate-treated corpses were the coleopteran *D. frischii*, conversely the diazinon corpse attracted three different species, two coleopterans, both members of the Histeridae family (*Euspilotus strobili* and *Hister spp.*), and *C. albiceps*; with *C. albiceps* being the very first colonizer. A plausible explanation can be that the aluminum phosphate ingested by the rabbit prior to death created an unsuitable environment for oviposition of *C. albiceps*.

It was also found that the species composition of the collected insects by order was significantly different in the diazinon-treated group compared to the other tested groups. Diazinon often attracted Coleopterans (especially Dermestidae family). While in the other tested groups, the dominant species were necrophagous dipterans belong to Calliphoridae

family.

In this study, a notable difference between the number and species of insects attracted to each corpse was detected, which can be useful in forensic entomology. Furthermore, all the tampered corpses experienced a more diverse range of colonizing necrophagous arthropods (especially in the aluminum phosphate-treated group), proving the hypothesis that the presence of these chemicals can affect insect diversity, with nortriptyline affecting both the number and the diversity of insects.

Based on the results of this study, it can be concluded that without considering the effects of chemicals that cause death, forensic entomology studies alone cannot be reliable. In all calculations and interpretations used in forensic entomology, especially for estimation of the postmortem interval (PMI), the effects of different chemical constituents and their metabolites on the behavior, abundance, succession, diversity, and development of forensically important arthropods in different geographical conditions should be considered. Therefore, it is suggested that similar comprehensive studies should be carried out in each region and the effects of most commonly used chemicals for suicide should be precisely determined to better understand the main role of these compounds and their metabolites on different aspects of forensically important insect's life cycle.

#### **Declarations**

**Funding:** Not applicable.

**Conflicts of interest/Competing interests:** No conflict of interest to declare.

**Consent to participate:** Not applicable.

**Consent for publication:** Not applicable.

**Availability of data and material:** All data are available.

**Code availability:** Not applicable.

**Ethical approval:** The project was done in accordance to the ethical principles and the national norms and standards for conducting Medical Research in Iran. The study was approved by Iran national Committee for Ethics in biomedical research (Approval ID: IR.SUMS.REC.1397.588).

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