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Study of alarm pheromones of Colorado potato beetles

Abstract. Study of pheromones of insects could give knowledge not only about ways of their communication and spreading information but also could be powerful tool to biologically control population of harmful insects that damage economy by destroying harvest. Colorado potato beetle *Lepinotarsa decemlineata* (Say) (*Coleoptera*: *Chrysomelidae*) is one of those insects that need to be controlled. Alarm and sex pheromones of the beetles were well studied by numbers of scientists around the world and even synthetic forms of them were synthesized. Meanwhile, alarm pheromones of Colorado potato beetles that are liberated during traumatic or stressful situations were not deeply studied and covered in relevant literature. This article describes experiments involving the beetles and attempts to collect alarm pheromones. Purpose of experiments were checking the effect of the pheromones on the beetles that are not under stressful conditions and running NMR-spectroscopy in order to compare results with spectrogram of aggregation pheromone that is described in the literature. The results showed that Colrado potato beetles responded with escape behavior when exposed to the extracted pheromones. NMR-spectroscopy analysis showed more signals and resemblance between the extract from abdominal parts of the beetles and aggregation pheromone.

Key words: Colorado potato beetle, Lepinotarsa decemlineata, pheromones, NMR spectroscopy.

Introduction

Pheromones are compounds produced and excreted to surrounding living organisms. They usually trigger specific responsiveness of identical species that dictates their behavior. They are synthesized in a small amount that brings some difficulties for chemical analysis. Fortunately, modern techniques of gas chromatography, mass-spectroscopy, and NMR-spectroscopy allow identifying even nanograms of any substance. Usually, pheromone is not an individual substance but a mixture of predominant component with some additives. These additives might be not active when applied separately but strengthen effect of the main ingredient in the mixture. Most of studied and identified pheromones consist of alcohols, their acetates, aldehydes and ketones.

Colorado potato beetle is an insect that damages harvest and reduces economic profit of agricultural sector. Through the years, some studies were done to investigate composition of their pheromones with a purpose of control population of the beetles. Sex pheromones that dictate copulatory behavior were studied and conclusion were drawn that male and female emissions have different compositions [1]. Other studies showed that aggregation pheromones are produced only by male species but attract both genders. The exact chemical composition of the pheromone was identified as (S)-1,3-dihydroxy-3,7-dimethyl-6-octen-2-one (1). A synthetic version of it was proven effective [2]. Moreover, an alternative way to synthesize the aggregation pheromone from geraniol was suggested [3].

There is another type of pheromones related to sending warning signals when an insect encounters stressful or dangerous situations. They are so-called alarm or fear pheromones. These signals can be monoterpenes, sesquiterpenes, or short-chain aliphatic hydrocarbons [4]. The responsive behavior for the alarm pheromone depends on the type of insects. Social insects tend to attack a source of threat, whereas non-social insects try to escape an uncomfortable area [5]. Literature review showed that alarm pheromones of Colorado potato beetles have not been studied. Meanwhile, synthetic form of these emissions could be used to biocontrol the population. The fact that alarm pheromones are the second most commonly produced pheromone used by insects, after sex pheromones make it even more appealing to study [6].

Materials and methods

Starting from mid-June till mid-July 2019, adult species and larvae of Colorado potato beetles were collected. They were sorted according to their genders and were kept in Petri dishes lined with potato leaves and dump cotton wool. Three chambers were built in order to collect volatile compounds excreted from the insects. The chambers had a rectangular shape with dimensions as 20 cm x 30 cm x 20 cm. The bottom and the roof were made of wood, the walls were made of plexiglass. Impermeability was secured with silicone glue and adhesive tape.

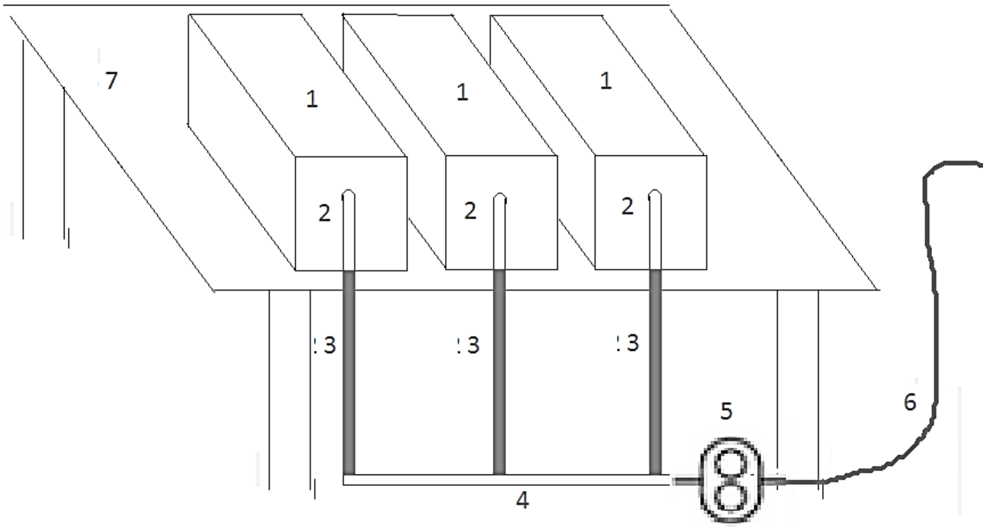

Figure 1 – System for collection of volatile compounds excreted from Colorado potato beetle.
Note: 1-chambers; 2-branch pipe; 3-columns filled with activated charcoal; 4-collector; 5-pump; 6-hose; 7 – table

Figure 1 represents a schematically drawn handmade system that was built specifically for a purpose of collection volatile alarm pheromones. On the side walls of the chambers there were holes at 15 mm height with branch pipes that were connected to columns filled with activated charcoal. A pump made it possible to push air with volatile compounds through the columns. After two days of keeping the Colorado potato beetles in the chambers, volatile compounds were extracted from activated charcoal by rinsing it with 100 ml of ethanol.

Thirty imago species were taken and dissected to three parts in order to identify an exact location of pheromone storage in Colorado potato beetles. Three parts were an abdomen, epicres, and the rest of a body with a head. The parts were collected respectively, put in a separate container. Each container then was filled with 50 ml of ethanol and kept in a dark cool place for seven days. After one week all three samples were filtered out and resulted filtrates were taken for NMR-spectroscopy at Shokan Ualikhanov Kokshetau University. NMR spectroscopy ^{1}H and ^{13}C were conducted by JNM-ECA Jeol 400 (frequency 399.78 and 100.53 MHz respectively) with a solvent $CDCl_3$. Chemical shifts were measured with regard to residual protons or carbon atoms of deuterated chloroform.

Results and discussion

The effect of extracted compounds that presumably contain alarm pheromones was studied on Colorado potato beetles. In order to check how the beetles in a state of dormancy would react to the extract, a drop of it was placed in front of the sleeping insect at approximately 1-2 cm distance. After 15 seconds, the beetle woke up and escaped from the drop to 5-10 cm distance. Next step was to check how the walking beetle would react to the extract. When a drop of the extract was placed in front of the walking insect at distance 1-2 cm, the beetle changed its walking direction. Another experiment included the beetle that was surrounded with the continuous drops of the extract in a shape of a circle with 10 cm diameter. Only one part of the circle was left empty as a «blind area» with a length 3 cm. Every beetle that underwent this experiment found the dry area and left the place through it. At last, a drop of the extract was placed directly on the insect. After that, the beetle fall into a stupor and died within some time. All the actions were repeated with water and ethanol. Water has not affected behavior of the insects at all; meanwhile there was a slight reaction to the effect of ethanol drops. However, the beetles have not tried escape from the area when facing ethanol drops. They tried to go away from them but not as fast as in the case of the extract. A direct contact with ethanol has not make them fall into a stupor; the beetles eventually got out of the discomfort zone.

It is obvious that alarm pheromones, which made the insects leave the uncomfortable area, change their route and even die as a result of direct contact, were present in the extract. Some studies related to alarm pheromones of insects and animals claim that their olfactory cells transform the semiochemicals into neural signals with unique properties that differ from those of canonical olfactory sensory neurons [5]. In some cases, species respond to alarm pheromones by joining together, showing aggression, and attacking a triggering object, in other cases individuals respond by dispersal. A behavior that was observed in the experiment clearly shows that Colorado potato beetles respond by escaping as a typical non-social clonal insect. There is not any evidence in literature review that direct exposure of alarm pheromone could be lethal to insects. Alarm pheromones generally consist of low molecular weight, highly volatile compounds that could easily spread throughout a colony yet evaporate quickly to terminate responsive behavior when traumatic stimulus no longer exists [7]. There is a possibility that components of the pheromone could chemically react with ethanol that was used for extraction. As a result of the reactions, other poisonous for the beetles products could be formed and cause fatal outcome after direct contact.

A model specter of ^{1}H NMR spectroscopy of a main component of the aggregation pheromone of Colorado potato beetle was obtained (Figure 2) and resulted spectrograms of ^{1}H NMR analysis were compared to it.

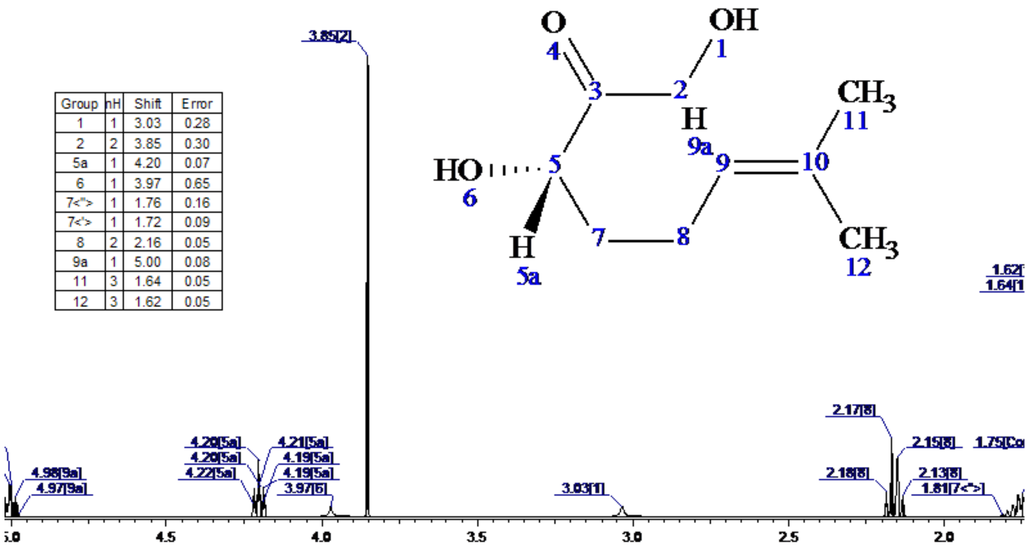

Figure 2 – A Model ^{1}H NMR spectrogram of a main component of Colorado potato beetle pheromone

A specter of a probe that contained extracts from the epicres had one absorption band at 1.56 m.d. that indicates presence of methylene protons H-11,11,11 and H-12,12,12, of the pheromone (Figure 3). Other signals were absent.

Figure 4 represents a spectrogram of 1H NMR spectrometric analysis of the extract from heads and bodies of the beetles. As it is seen, there are more protonic signals identical with the model spectrogram. However, there are more specters than anticipated. Chemical shifts at 1.24-2.29 m.d. that are characteristic for methylene protons H-11,11,11 and H-12,12,12 of the model pheromone are suitable for qualitative identification of the pheromone.

A signal that is seen at 1.59 m.d. correlates with methylene protons H-7,7. A chemical shift at 2.28 m.d. is comparable to a proton H-8,8, in the model pheromone. A shift at 3.30 m.d. can be interpreted as a hydroxyl proton H-1. A singlet signal at 4.14 m.d. correlates with a model proton H-5.

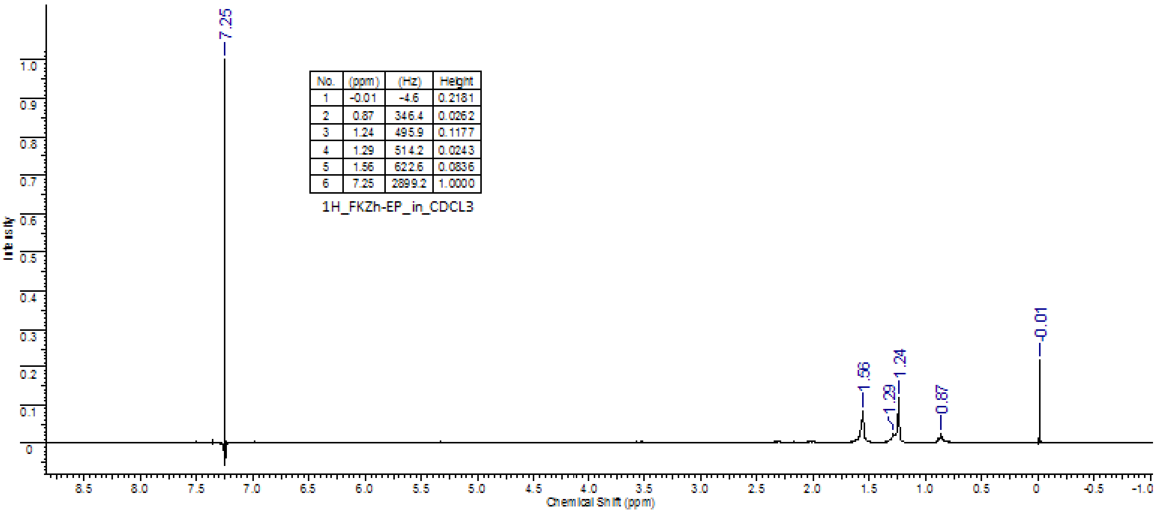

Figure 3 – NMR-spectrogram of an extract from epicres of Colorado potato beetle

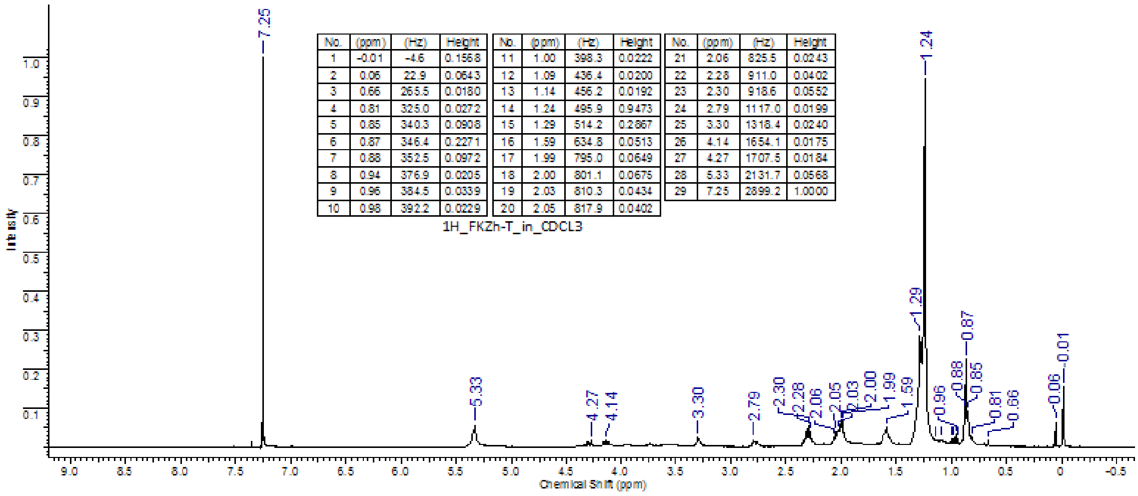

Figure 4 – NMR-spectrogram of an extract from bodies and heads of Colorado potato beetles

A spectrogram obtained from ^{1}H NMR spectroscopy of the extract from abdominal parts of Colorado potato beetles has chemical shifts that corresponds with the model spectrogram as well (Figure 5).

Figure 6 represents a model ^{13}C NMR spectrogram of a main component of Colorado potato beetle pheromone and obtained ^{13}C NMR spectrograms of the extracts from abdominal parts, heads and bodies, epicres. As it is seen, NMR spectrograms were not successful; only signals from the solvent $CDCl_3$ is present. This could occur for several reasons, for instance, many components in the mixture have shielded each other.

The beetles that were taken for experiments underwent through stressful and traumatic conditions and were expected to release alarm pheromones. An assumption was made that pheromones consist of mixture of components and their quantitative ratios depend on the required response from the same species. As NMR-spectroscopy results showed well-studied in the related literature main component of aggregation pheromone could be present in the extracts of different body parts of the beetles. The most closest and abundant signals were seen from the spectrogram of the extract from abdominal parts. Extracted pheromones from different parts of Colorado potato beetles corresponds to pheromones from the literature review. The only result that did not show full resemblance is the NMR spectrogram of the extract from epicres of the beetles. There is a possibility that epicres of thirty beetles were not enough or aggregation pheromones has a different structure. It was assumed that alarm pheromones of Colorado potato beetles might be released and stored in the malpighian tubes. As literature shows, alarm pheromones of eusocial insects like ants and bees have been profoundly studied and results show that these chemical releasers are produced in glands and stored in reservoirs which could be dissected from the insect [8]. As it was mentioned before eusocial insects show aggressive and defensive response to release of alarm pheromones. Colorado potato beetles showed completely different escape behavior to traumatic stimulus. The different response could be explained with unique to each species mechanism of information transmittance in neurons.

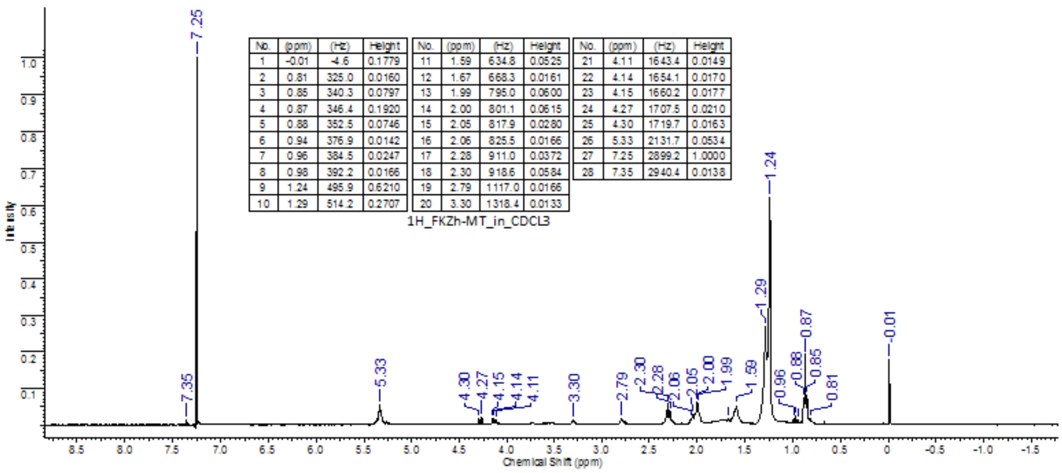

Figure 5 – NMR-spectrogram of an extract from abdominal parts of Colorado potato beetles

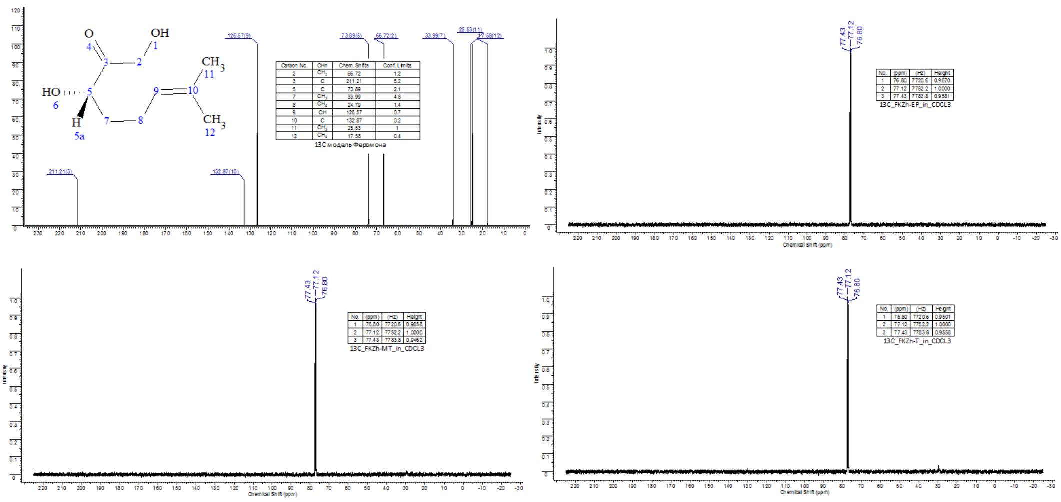

Figure 6 – A model spectrogram of ^{13}C NMR spectroscopy of the main pheromone component and NMR-spectrograms of the probes

Conclusion

Alarm pheromones of Colorado potato beetles is not covered in related literature, meanwhile sex and aggregation of the insects are well studied and even artificially synthesized. The first experiment that was carried out by authors involved keeping the beetles in uncomfortable confined space, so they experience traumatic stimulus triggering them to release alarm pheromones. The pheromones were adsorbed, extracted and its effect was checked on Colorado potato beetles in different states. In all cases the insects showed escape behavior that is relevant for non-social species. A direct contact of drops of the extract of the pheromones with the beetles led to death of the insects. The later phenomenon could be used to biocontrol population of Colorado potato beetles. However, more deep studies should be done to understand chemical composition of alarm pheromones and methods for extraction, handling and storage as well as their chemical properties. An attempt to identify an organ where alarm pheromones are produced and stored was made. ^{1}H NMR-spectroscopy of extracts of different body parts of the beetles showed that the most abundant and closest signals compared to spectrogram of aggregation pheromone was observed in spectrogram of the extract of abdominal parts of the beetles. An assumption was made that the alarm pheromone consist of mixture of compounds and has a main component of well-studied aggregation pheromone which spectrogram was used as a model to identify main chemical shifts. More elaborated procedure of chemical analysis is required to determine qualitative and quantitative composition of alarm pheromone of Colorado potato beetles. Probably it is better to separate the mixture with a chromatography technique initially and couple it with mass spectrometry to identify chemical structures of constituents.

References

1 Dubis E., Malinski E., Dubis A., Szafranek J., Nawrot J., Poplawski J., and Wrobel J.T. (1987). Sex-dependent composition of cuticular hydrocarbons of the Colorado beetle, *Leptinotarsa decemlineata* (Say). *Comp. Biochem.*, vol. 87, no. 4, pp. 839–843. https://doi.org/10.1016/0300-9629(87)90002-8.

2 Dickens J., Oliver J.E., Hollister B., Davis J.C., Klun J.A. (2002). Breaking paradigm; male-produced aggregation pheromone for the Colorado potato beetle. *J. Exp. Biol.*, vol. 205, no.13, pp. 1925-1933.

3 Wu Z., Jager M., Buter J., Minnaard A.J. (2013). A protecting group-free synthesis of the Colorado potato beetle pheromone. *Beilstein J. Org. Chem.,* vol. 9, no. 1, pp. 2374-2377. https://doi:10.3762/bjoc.9.273.

4 Ginzel M.D. (2010). Encyclopedia of animal behavior. Cambridge: Academic Press. pp. 584-588. ISBN 978-0-08-045337-8.

5 Enjin A., Sun G.S. (2013). Neural mechanisms of Alarm Pheromone signaling. *Mol.Cells,* vol. 35, no. 1, pp. 177-181. https://doi/10.1007/s10059-013-0056-3.

6 Vandermoten S., Mescher M.C., Francis F., Haubruge E., Verheggen F.J. (2012). Aphid alarm pheromone: an overview of current knowledge on biosynthesis and functions. *Insect Biochem. Mol. Biol.*, vol. 42, no. 3, pp. 155-163. https://doi.org/10.1016/j.ibmb.2011.11.008.

7 Klowden M.J. (2013). Physiological system in insects. Cambridge: Academic Press. pp. 603-647. ISBN 9780124158191.

8 Mizunami M., Yamagata N., Nishino H. (2010). Alarm pheromone processing in the ant brain: an evolutionary perspective. *Front. Behav. Neurosci.*, vol. 4, no. 28, pp. 1-9. https://doi: 10.3389/fnbeh.2010.00028.