Agricultural Sciences, 2023, 14, 499-508
https://www.scirp.org/journal/as
ISSN Online: 2156-8561
ISSN Print: 2156-8553

Pheromone Monitoring in the Granaries of Uzbekistan

Gulnara Shakirzyanova*, Abdusamat Nabiev, Omon Kholbekov, Vladimir Abdukakharov

Laboratory of Low Molecular Weight Biologically Active Compounds, Institute of Bioorganic Chemistry Named after Academician A.S. Sadykov Uzbek Academy of Sciences, Tashkent, Uzbekistan
Email: *gulnara-sh@rambler.ru

How to cite this paper: Shakirzyanova, G., Nabiev, A., Kholbekov, O. and Abdukakharov, V. (2023) Pheromone Monitoring in the Granaries of Uzbekistan. *Agricultural Sciences*, **14**, 499-508.
https://doi.org/10.4236/as.2023.144033

Received: February 20, 2023
Accepted: April 16, 2023
Published: April 19, 2023

Abstract

The pheromonitoring of pest insects population was carried out in the granary conditions. The results of pheromonitoring let us detect the pest insect's location at lowest abundance, while visual inspections are not effective, and to determine the data of effective location in granary, proportions traps and pheromone preparative dose. The aggregation pheromone of the synthetic analogue *Sitophilus* weevils had been carried out, according to the general procedure for preparation of aldol product. Especially for increasing attractiveness of pheromone traps, different types of food baits were tested. The results were observed with using of germinated wheat grains moisten by wheat germ oil. The corn oil was more attractive and effective for attractiveness of whole pest insects in granary. The wheat germ oil and burdock oil was preferred, as food bait, especially for weevils. The special perforated construction of the pheromone trap for pest insects usage has been developed and recommended for storage areas. In a condition of granary, successful testing of pheromone monitoring makes it possible to establish the timing and necessity of quantitative chemical treatments.

Keywords

Pheromone, Monitoring, Pheromone Trap, Granary Pest Insects

1. Introduction

One of the main factors for growing high quality crops and preserving agricultural products are timely manner and effective protection from pest insects and their diseases. In the world, more than 200 million tons of grain, about 25 million tons of vegetables and more than 11 million tons of fruits per year are lost because of vital activity of pest insects. In connection with increasing needs of the population for ecologically and organic agricultural products, a huge role is given to the integrated system of protection against pest insects, which combines chemical and biological methods [1].

In the world, pest management technology has been widely used for monitoring and controlling insect pests in agriculture. It became a popular tool in developing environment-friendly control technologies for integrated pest management [2].

It is very actual to protect grain and grain's processing products in a period of their storage. In granaries, grain protection from pest insects was conducted by using chemical methods. The goal of such treatment is extermination of insects. During growing, the insects excrete waste products in storage grain, which influences grain quality. The insects feed on grain, eat out the endosperm, and all that process negatively affects the yield of flour. During grain storage, grain impurities and loss of grain increase. The insects emitted excrement and their respiration increased the grain temperature and humidity, which led to self-heating and damaging of the grain [3].

Pathogens, weeds, and invertebrates cause significant crop losses worldwide, while representing an obstacle to achieving global food security and poverty reduction. The usage of synthetic pesticides presents additional challenges and new alternative methods, which could reduce pest damage and negative outcomes associated with synthetic pesticides. Integrated Pest Management (IPM) represents different methods of pest controlling and promotes reducing the application of synthetic pesticides [4].

It is very perspective to use natural bio-regulator pheromones of pest insects in the system of protective measures.

Pheromones are natural chemicals emitted, in many insect species, by females to attract males for mating, for insects aggregation, for danger signaling and other signaling vital reflexes. These compounds are extremely potent, essentially non-toxic, and species-specific. The mechanism of natural pheromones is complex and varies depending on the type of pheromone and the species that is producing it. However, in general, natural pheromones work by binding to specific receptors in the nose or mouth of the receiving animal. This binding triggers a series of biochemical reactions that ultimately lead to a behavioral response.

The usage of pheromones in granaries, let to protect the best quality of stored grain and at the same time to avoid environmental pollution. Synthetic analogues of sex pheromones have proven themselves well specifically for the detection and monitoring of insect pest populations in food storage conditions.

In granaries, the system of protection measures supposes the usage of toxic chemicals. And many of that chemicals are highly toxic for humans and warm-blooded animals. Fumigation of large-scale storage granaries by the application of synthetic chemicals such as methyl bromide and aluminum phosphide has been used to control storage pest species [5].

Therefore, the searching of safer protection means is a very actual question. Also, the searching of new highly effective pesticides against the pest insects is dictated by the increasing of living organisms resistance to constantly usage of chemicals, and also to lead for appearance of resistant pest insects populations.

In the midst of the chemicals, and pesticides with biocidal activity there are functional analogues of semiochemicals—natural substances, which could play the role of signaling molecules. Such natural compounds are responsible for controlling the main life processes both in the life of individual organisms and in intrapopulation, intraspecific and interspecific relationships of arthropods.

Among such drugs, which radically differ in the mechanism of action from most of the used pesticides with biocidal activity, are compounds created on the basis of functional analogues of semiochemicals—natural substances that play the role of signaling molecules responsible for controlling the main life processes in the individual organisms and in intrapopulation, intraspecific and interspecific relationships of arthropods. The main role of natural bioregulators of arthropods belongs to pheromones, juvenile hormones, their analogues, etc.

Recently, pheromones have been used in the world as one of the main components of system complex for protection of storage products. By using pheromone traps, the dates of insect infestation of storage products, determination of the pest insects phenology, the beginning of their highest activity and time of their flying are obtained, and after that, system of fight against them is organized. On the other hand, pheromone traps are used for mass trap catching of pest insects. As a result of the use of pheromone traps, pheromonitoring of the number of insect pests was carried out, which makes it possible to reduce the cost of purchasing insecticides and improve the quality of stored products. Using the results of pheromonitoring allows you to identify the location of insect pests in conditions of the smallest number, when visual inspections are ineffective. Based on the results of pheromonitoring under storage conditions, data on the effective placement in the granary, the proportions of traps, and the preparative dose of pheromone are determined. In the conditions of granaries, the successful development of pheromone monitoring makes it possible to establish the timing and the need for quantitative chemical treatments [6].

The main goal of our research was to carry out pheromonitoring in a granary, determine the preparative dose of weevil aggregation pheromone and develop an effective design of a pheromone trap for use in food storage conditions.

2. Study Area

Pheromonitoring was carried out in the granary of warehouse "G'ALLA-ALTEG", Tashkent, Uzbekistan. Monitoring was carried out according to the standard methodology [7].

In Uzbekistan, the main dangerous weevils of the genus *Sitophilus* are pests of stored grain, such as the flour destroyer beetle (*Tribolium destructor*), flour moth (*Ephestia kuehniella*) and others. Potentially dangerous insect pests in granaries include granary weevil, rice weevil, destroyer flour beetle, Suriname flour beetle, red flour beetle, southern granary and grain (cocoa) moth. Monitoring of these species should be given the most attention [8].

3. Materials and Methods

Chemicals and solvents were purified by standard techniques. For thin-layer chromatography (TLC), silica gel plates Silufol, eluents (ethyl ether-hexane/1:1), compound were visualized by irradiation with iodine vapor. Flash chromatography was performed using silica gel Merck 60 F254, eluents—system of ethyl ether and hexane.

HPLC was carried out using an Agilent 1100 Series, USA, using chiral column—Amylose tris [(S)-*α*-methylbenzylcarbamate], covered silica gel 10 μm (ChiralPak®AS 0.46 см × 25 cm, DAIC 20025, Daicel Chemical Industries, LTD, France), fluid phase A—ACN and B—*i*-PrOH in gradient mode: 0 - 10 min B2% - 10% and 10 - 15 min B10%.

High-resolution mass spectra were recovered on 6420 Triple Quad LC/MS (Agilent Technologies, USA) Eluents—acetonitrile in isocratic regime. Flow rate – 0.25 mL/min. Registration of mass-spectra was conducted with negative ionization. The scanning range—50 - 2200 m/z, dehydrating gas consumption—3 L/min, gas temperature 300˚C, gas pressure on needle sprayer 20 psi, evaporator temperature—300˚C, tension on the capillary—4000B.

General procedure for preparation of Aldol product

According to [9], a heterogeneous mixture of anhydrous chloroform (10 mL) and 3-pentanone (0.06 mol) and L-proline (25% mol) was added to the propionaldehyde (3 mmol). The resulting mixture was stirred at room temperature for 32 h. The reaction mixture was treated with saturated NH_4Cl solution, the layers were separated, and the aqueous layers were extracted several times with ethyl acetate, dried with anhydrous Na_2SO_4, and evaporated. The pure aldol product was obtained by flash column chromatography. Yield: 74%. HPLC (ChiralPak® AS – *Amylose tris* [(*S*)-*α-methylbenzylcarbamate*], 98% MeCN—2% i-PrOH t_R (major) = 7.998 VP, t_R (minor) = 7.072 VV; S:R—57.0722:28.0855, t_R (major) = 8.007 VP, t_R (minor) = 7.025 VV; S^*:R^*—43.4765:30.0490.

MS [$C_8H_{16}O_2$], 143 $[M – H^+]^–$, 87 $[M – C_3H_5O]^–$.

Monitoring weevils in a granary conditions

Pheromonitoring studies were carried out in the granary of warehouse "G'ALLA-ALTEG", Tashkent, Uzbekistan.

For our experiments, we used a bunker with grain in the amount of about 6000 tons. Pheromone traps in the amount from 1 to 6 (including 7c (control traps)) were laid out along the most heated wall of the granary (sunny side). The traps were set in the upper surface layer, and only the upper protective layer was visible from the seeds, 5 - 10 cm from the edge. Traps with number from 8 to 10 (including 14c) were laid out along the longitudinal axis of the warehouse in the upper surface layer. Traps with number from 11 to 13 (including 15c)—along the longitudinal axis of the warehouse, buried to a depth of 1 m.

After some time, the pheromone traps were removed from the mass of grains, their contents were analyzed. On the base of the results, conclusions were made about grain damageability by pest insects.

The results of experiments are represented in Table 1.

According to the experiments, the best result was obtained in the pheromone trap—up to 173 weevils, located in the upper grain layer, near the most heated wall of the granary. One of the control 7c traps, without pheromone, observed 7 individuals weevils, which could be accidentally caught. Moreover, in the 15c trap—there were no insects at all.

We conducted tests, especially for comparing our new design pheromone trap with analog traps. Special perforated construction, supplied with pheromone capsule, food bait and laminated double-sided insert with adhesive surface and open surface with the same composition were compared in conditions of granary. The results of experiments are represented in Table 2 and Table 3.

Table 1. Monitoring weevils in granary conditions.

Pheromone trap Special perforated construction	Pheromone preparative dose, mg	Food bait	Quantity of pest insects in trap (duration 2 weeks)
1	0.99	germinated wheat grains	**103-weevils (W)** 9-other granary pest insects, caterpillars **3 alive weels**
2	0.99	germinated wheat grains	**52-weevils (W)**
3	0.99	germinated wheat grains	**173-weevils (W)**
4	0.99	germinated wheat grains	**18-weevils (W)**
5	0.99	germinated wheat grains	**73-weevils (W)**
6	0.99	germinated wheat grains	**110-weevils (W)** 17-other granary pest insects
7(c)	-	entomological glue	**7 weevils (W)** + Psocoptera
8	0.99	germinated wheat grains	**8-weevils (W)**
9	0.99	germinated wheat grains	**77-weevils (W)** 6-other granary pest insects
10	0.99	germinated wheat grains	**6-weevils (W)** 3-other granary pest insects
11	0.99	germinated wheat grains	**3-weevils (W)** 9-other granary pest insects
12	0.99	germinated wheat grains	**41-weevils (W)**
13	0.99	germinated wheat grains	**15-weevils (W)** 5-other granary pest insects
14(c)	-	germinated wheat grains	**15-weevils (W)**
15(c)	-	entomological glue	-

Table 2. Comparative the catchability estimation of the proposed pheromone trap and analogue trap for pest insects of storage product (duration one month).

Type of trap	Attracted insects, (pieces)			
	total		for 1 pheromone trap	
	weevils		other granary pest insects	Psocoptera
Special perforated construction	9	1	259	Psocoptera
Analog, open surface	6	1	110	Psocoptera

Table 3. Comparative estimation of attractiveness proposed pheromone trap and analogue trap for pest insects of storage product (duration one month).

Type of trap	Attracted insects, (pieces)			
	total		for 1 pheromone trap	
	weevils		other granary pest insects	Psocoptera
Special perforated construction	83	12	136	-
Analog, open surface	69	8	103	-

According to the obtained results the special perforated construction of the pheromone trap has more efficiency in attracting insects in comparison with analogue traps.

4. Results and Discussion

4.1. The Aggregation Pheromone of Weevils

The aggregation pheromone of *Sitophilus granarius* and *Sitophilus oryzae* are produced by the insects during a period favorable for the development of a population, that is, where there is sufficient food and breeding opportunities, as well as appropriate environmental humidity and ambient temperature. The food attractants and sexual stimulants cannot supersede the aggregation pheromone produced by both females and males. The rice and corn weevils were found to produce the aggregation pheromone in the form of (4S,5R)-hydroxy-methyl-3-heptanon-eenantiomer (**Figure 1**) [10].

We have synthesized diastereomers of 4-methyl-5-hydroxy-heptan-3-one in different methods: at the first—in the conditions of aldol condensation between diethylketone and propylaldehyde, corresponding ketol has been received [11], and also the asymmetric catalytic aldol reaction was carried out according to interaction of 3-pentanon and propylaldehyde by L-proline initiation.

In the conditions of aldol condensation between diethylketone and propylaldehyde, we have received the corresponding ketol—4-methyl-5-hydroxy-heptan-3-one, which was relatively called Sitofilure—aggregation pheromone of granary and rice weevils (**Figure 2**).

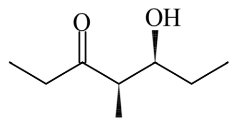

Figure 1. (4S,5R)-hydroxy-methyl-3-heptanone.

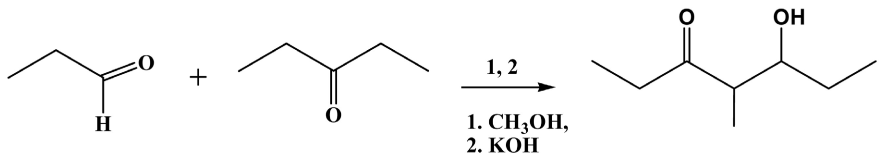

Figure 2. Scheme the aldol condensation between 3-pentanon and propylaldehyde.

The optimal conditions for aldol condensation—1.33 m 3-pentanon, 0.33 m propylaldehyde and 7% KOH solution in methanol. Temperature reaction 15˚C. Yield: 50%. The ratio of R*S* and R*R*—diastereomers was about 1:1.

We also implemented synthesis of aggregation pheromone weevils, in a conditions of aldol addition in a presence of L-proline as a catalyst.

In the result of interaction 3-pentanone, as a donor and propionaldehyde, as a acceptor into the presence of L-proline, direct asymmetric aldolization have been conducted. L-proline could catalyze asymmetric aldol addition into conditions of anhydrous solvents. Reaction is conducted via an enamine intramolecular complex. Intramolecular complex of direct asymmetric aldol reaction represents enamine formation between L-proline and appropriate 3-pentanone—donor-substrate. After that, acceptor's carbonyl-group from propionaldehyde attactes enamine intramolecular complex and the process had completed by formation of aldol product (**Figure 3**).

The chloroform was more suitable solvent for cross-coupling. Aldolization went via an enamine intramolecular complex. Changing terms of reaction couldn't influence on product's enantioselectivity. After separation reaction product was analyzed by HPLC using (ChiralPak® AS – *Amylose tris* [(*S*)*-α-methylbenzylcarbamate*], 98% MeCN—2% i-PrOH.

Also, we have analyzed component consisting of **(4S,5S)**-4-methyl-5-hydroxy-heptan-3-one by ESI-MS. Registration of ESI-MS were conducted with negative ionization.

4.2. Pheromonitoring in the Conditions of Granary

Pheromonitoring was carried out in the granary of warehouse "G'ALLA-ALTEG", Tashkent, Uzbekistan, according to the methodology [7].

Pheromone traps were laid out along the most heated wall of the granary (sunny side). Some pheromone traps were placed into the upper surface layer and only the upper protective layer was visible from the seeds. And some pheromone traps were laid out along the longitudinal axis of the warehouse in the upper surface layer. And last of them—along the longitudinal axis of the warehouse, buried to a depth of 1 m.

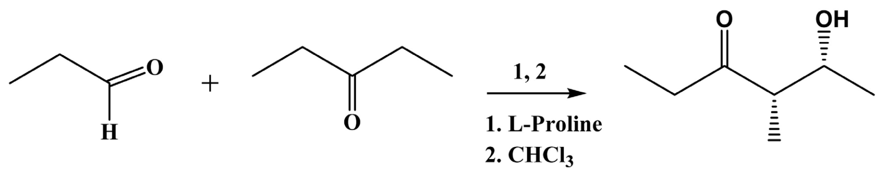

Figure 3. Scheme L-proline catalyzed asymmetric aldol reaction.

On the base of our experiments the preparative pheromone dose was obtained. According to our results, the highest pheromone dose in a perforated container had repellant properties. The presence of food bait in the trap helped to attract other granary pest insects into the traps.

4.3. Study of Different Varieties of Food Baits for Pheromone Traps

During our experiments, we studied the different varieties of baits for pheromone traps. We conducted the attractiveness analyses of vegetable oils to attract pest insects in granaries [12].

We have tested some different oils: sunflower, corn, olive, wheat germ oil and burdock oil. At the control was used entomological glue. Based on our experiments in the granary, corn oil was more attractive and effective in attracting whole insect pests insects—beetles and butterflies. According to our tests, the insects species belonging about to 8 families were detected in pheromone traps with corn oil. After the corn oil, the next attractive oil was wheat germ oil and burdock oil. This, apparently, indicates that the reason for the different selectivity of vegetable oils in relation to insect pests is due to the different biochemical composition of oils.

4.4. Designs Varieties of Pheromone Traps

There are a lot of different pheromone traps designs, which are used for detection and monitoring of the pest insects population, and to control their quantity through the mass males capturing. The set of typical pheromone trap consists of pheromone evaporator, food bait and glue laminated liners. It should be noted that, there are some limits for usage of pheromone traps, because of only adults of pests insects are attracted to pheromone traps, and larvae of different ages often cause more damages for grain and grain products. On the other hand, pheromone traps let to obtain abundance of information about the pest insects and causes to develop protective measures [13].

We developed and patented specifically construction of pheromone trap for pest insects for usage in places of granary [14]. The pheromone trap has hollow perforated container with pheromone capsule, food bait and laminated insert with a double-sided adhesive surface.

It should be noted that weevils are mainly localized in the upper layers of the grain mound, and it was the most favorable condition for their living.

The proposed design—special perforated construction of the pheromone trap, in comparison with analogue traps, ensured high efficiency of insects attractiveness. Our results, in condition of granary, showed the greater efficiency of insect attractiveness of special perforated construction of the pheromone trap in comparison with the analogue traps.

5. Conclusions

The aggregation pheromone of the synthetic analogue *Sitophilus* weevils had been carried out, according to the general procedure for preparation of aldol product. The preparative pheromone dose was obtained in a condition of granary.

It should be noted that in the granary, weevils are mainly localized in the upper layers of the grain mound, and it was the most favorable condition for their living.

Various food baits have been tried specifically to increase the attractiveness of pheromone traps. And the best results were observed with using of germinated wheat grains moisten with wheat germ oil. At the same time, it was found that corn oil was more attractive and effective in attracting whole insect pests to the granary. Wheat germ oil and burdock oil were preferred as food bait for weevils.

Based on our research, we can recommend special perforated construction of the pheromone trap to fight pest insects in storage areas.

Such wise, pheromonitoring, successfully used in storage areas, has significantly reduced the deterioration of storage products from infection by pests.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] FAO (2022) Crop Prospects and Food Situation—Quarterly Global Report No. 4, December 2022. https://doi.org/10.4060/cc3233en

[2] Cui, G.Z. and Zhu, J.J. (2016) Pheromone-Based Pest Management in China: *Past, Present, and Future Prospects*. *Journal of Chemical Ecology*, **42**, 557-570. https://doi.org/10.1007/s10886-016-0731-x

[3] Burov, B.N. (2000) Pheromone Traps in the Granary. *Protection and Carantine of Plants*, No. 7, 15-16. (Russian Version)

[4] Pretty, J. and Bharucha, Z.P. (2015) Integrated Pest Management for Sustainable Intensification of Agriculture in Asia and Africa. *Insects*, **6**, 152-182. https://doi.org/10.3390/insects6010152

[5] Sousa, A., Faroni, L., Pimentel, M. and Guedes, R. (2009) Developmental and Population Growth Rates of Phosphine-Resistant and Susceptible Populations of Stored-Product Insect Pests. *Journal of Stored Products Research*, **45**, 241-246. https://doi.org/10.1016/j.jspr.2009.04.003

[6] Smetnik, L.I., Kuzina, N.P. and Kovalev B.G. (1987) Methodical Recommendation for Detection of Quarantine Pest Insects by Using Pheromone Traps, Moscow, Gosagroprom USSR, 1-11.

[7] GOST 12045-97 Interstate Standard (1998, July 1) Seeds of Agricultural Crops, Me-

thods for Determining the Population of Pests (Russian Version).

[8] Zakladnoy, G.A., Mortgage, G.A. and Ryazantsev, M.I. (1980) Distribution of Insects in the Grain Mass and Their Detection Using Traps, Sat. scientific tr., VNIIZ., Issue 93, 28-41.

[9] Shakirzyanova, G.S., Romanova, L.Ch., Babaev, B.N., Abdukacharov, V.S., Iskandarov, T.I. and Gaibova, S. (2021) Synthesis of Synthetic Analogue Sitophilus Weevils Aggregation Pheromone and Studying of Hygienic and Toxicological Indexes. *Acta agriculturae Slovenica*, **117**, 1-8. https://doi.org/10.14720/aas.2021.117.2.1961

[10] Faustini, D.L., Giese, W.L., Phillips, J.K. and Burkholder, W.E. (1982) Aggregation Pheromone of the Male Granary Weevil, *Sitophilus granarius* (L.). *Journal of Chemical Ecology*, **8**, 679-687. https://doi.org/10.1007/BF00988309

[11] Fauve, A. and Vaschambre, H. (1987) Use of Biological Systems for the Preparation of Chiral Molecules IV: A Two Step Chemoenzymatic Synthesis of a Natural Pheromone (4R,5S)-(−)–4-Methyl, 5-Hydroxy Heptan-3-One, Sitophilure. *Tetrahedron Letters*, **28**, 5037-5040. https://doi.org/10.1016/S0040-4039(00)96689-9

[12] Pimenov, S.V. (2003) Identification of Pests of Food Stocks with the Help of Oil Attractants, Materials of the 68th Scientific-Practical Conference "Problems of Ecology and Plant Protection in Agriculture"—Stavropol, 165-168. (Russian Version)

[13] Sazonov, A.P. (2000) Pheromone Traps at Bakery Enterprises. Protection and Quarantine of Plants, N 7, WITH, 15-16. (Russian Version) https://www.elibrary.ru/contents.asp?id=34082097

[14] Shakirzyanova, G.S., Nabiev, A.Kh. and Salikhov (2020) Patent FAP 014666, 01/22/2020. "Pheromone Trap for Insects", Approved by the Intellectual Property Agency of the Academy of Sciences of the Republic of Uzbekistan.