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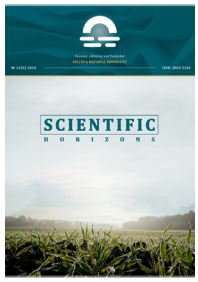

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Synergism of a mixture of phosphine and carbon dioxide in fumigation against bean weevils

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Abstract. The study is devoted to searching for alternatives to methyl bromide – a universal fumigant restricted in use at the request of the Montreal Protocol. Purpose: to determine the synergy of a mixture of phosphine and carbon dioxide in their various concentrations and exposures during the fumigation of leguminous products against pests at high temperatures. Methods: analytical review on the subject of research, the current regulatory framework in the field of disinfection; analysis of biological features of insect pests of leguminous products; experimental – a variation of different concentrations of phosphine, duration of exposure and temperature to establish a synergy of fumigant with carbon dioxide in laboratory conditions with appropriate equipment; mathematical and statistical – using computer mathematical functions built into the Microsoft Excel program 2003. When fumigating gas mixtures against pests at the imago stage, the optimal concentration of carbon dioxide is in the range of 110-130 g/m^3 (or 5.5-6.5% of the total air volume), regardless of the temperature indicator. While the optimal concentration of phosphine at low temperatures (21-22°C) is in the range of 0.57-0.82 g/m^3, at high temperatures – (31-32°C) – in the range of 0.21-0.36 g/m^3. With an increase in the duration of fumigation, not only the effectiveness of phosphine (standart) against the imago pests but also gas mixtures. In addition, the dependence of gas synergy on the duration of fumigation was established. Thus, at a temperature of 30°C, an increase in gas synergy in the mixture was observed by 4.4±0.66, 7.7±1.61, and 10.3±1.08% compared to the standard for exposure of 2, 4 and 6 hours, respectively. Temperature was the most determining factor influencing the value of the gas synergy index in the mixture of phosphine and carbon dioxide. Thus, an increase in temperature from 30°C to 32°C contributed to an increase in the gas

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synergy index by 9%, that is, by 2.17 times. Further establishment of lethal standards and fumigation regimes against pests, including quarantine ones, can prevent economic damage from these pests in Ukraine and will contribute to the implementation of the decisions of the Montreal Protocol aimed at protecting the environment and preventing the destruction of the ozone layer

Keywords: alternative to methyl bromide; concentration; duration of exposure; temperature; pests

INTRODUCTION

The problem of preserving crops from pests of leguminous stocks, as well as preventing economic losses from the penetration and spread of regulated quarantine species in Ukraine is extremely important. The quarantine pests of leguminous products that can enter and acclimatise on the territory of Ukraine are Chinese (Chinese *Callosobruchus chinensis* L.) and cowpea weevils (*Callosobruchus maculatus* Fabre).

Given the fact that these organisms are pests of stocks and are already present on the territory of European countries, the probability of their entry and acclimatisation on the territory of Ukraine is quite high. Therefore, as of 2021, the distribution area of *C. chinensis* occupies 7 countries in Europe, in particular, the Czech Republic and Bulgaria (CABI digital library, 2021). Furthermore, A. Singh, T. Boopathi (2022) indicate a high breeding potential of the pest. They prove that the relative growth rate of *C. chinensis* can be 0.101±0.006 mg/day. Kébé Khadim *et al.* (2017) claim that the spread of *C. maculatus* is related to the trade of its main host plant *Vigna unguiculata*. Kalpna *et al.* (2022) note that these pests can cause up to 20% of crop losses during storage. Price *et al.* (2017) during the studies, observed the adaptation of *C. maculatus* to 6 new host plants.

Fumigation in the plant protection and quarantine system is one of the most effective radical ways to control pests. Wöhr & Frey (2020) developed a detailed algorithm for limiting the use of methyl bromide until it is completely stopped. Among the existing fumigants that could be used as a substitute for methyl bromide, the choice is very limited. The most common is phosphine. Yet along with its relative safety in use, this fumigant has a number of disadvantages, in particular, pest resistance to phosphine. Thus, Nayak *et al.* (2020) established that the problem of pest resistance has worsened over the past two decades, mainly due to the lack of suitable alternatives that meet the main characteristics of phosphine, including its low price, ease of use, and compatibility with most storage conditions.

In addition, the problem is aggravated by the fact that only one fumigant is registered in Ukraine – phosphine, or its solid preparative forms with two active substances: aluminium phosphide or magnesium. Resistance of some pests to phosphine is not uncommon, which is noted by researchers. Thus, Konemann *et al.* (2017) indicate that the level of resistance in the most persistent populations of *Cryptolestes ferrugineus* was 133.5 times higher than in the sensitive laboratory population. Holloway *et al.* (2016) prove that 24 populations of *Sitophilus oryzae* were diagnosed as resistant to phosphine in Australia.

In recent years, studies have been actively conducted with mixtures of various gases to establish their possible synergy against harmful organisms. Rajendran & Somiahnadar (2020) indicate that the success of pest control lies in the individual approach and the use of several methods and/or combined treatment methods, including mixtures of gases. For example, Klechkovsky & Neamtsu (2020) prove that quarantine treatment by fumigation of fresh potato tubers with a mixture of carbon dioxide and methyl bromide against the quarantine pest – potato tuber moth is possible with the consumption rate of CH_3Br 4 times lower than its dosage applied in its pure form.

Cho *et al.* (2020) show that fumigation with ethyl formate mixtures with a concentration of 16 mg/L and PH_3 0.1 mg/L for 4 hours, can ensure the complete death of imago and nymphs of these pests. However, Kwon *et al.* (2023) focus on the fact that the phytotoxic effect of the examined gas mixtures on live plants was considerably lower, in contrast to brommethyl.

There are data on the insecticidal effect of essential oils (an alternative to the chemical method) against *C. maculatus* and *C. chinensis*. Thus, Gupta *et al.* (2023) note that the action of extracts from such plants as *Acorus calamus, Lavandula angustifolia*, and *Cedrus deodara* against *C. chinensis* at parameters of 92.18-118.54 μL/L, and *A. calamus, L. angustifolia and Pinus wallichiana* against *C. maculatus* at parameters of 204.01-312.23 μL/L, ensured 50% death of pests.

Gad *et al.* (2021) observed 100% efficiency of ozone exposure in the imago stage of these pests. However, the inactive stages (eggs and pupae) were stable. Weining Cheng *et al.* (2013) conducted experiments under two modified atmospheric conditions: (1) 2% O_2+18% CO_2+80% N_2 and (2) 2% O_2+98% N_2. Both hypoxic environments substantially affected the development and survival of all stages of the development of *C. maculatus*. Manar *et al.* (2021) present the results of studies on the 100% effectiveness of fumigation with ECO2FUME against these pests. In addition, the germination rate of fumigated legume seeds was higher than in the control group. However, the researchers did not determine the effectiveness of synergy of mixtures.

Thus, the purpose of this study is to examine the toxic effect of a mixture of phosphine and carbon dioxide and their synergy against pests under various fumigation parameters.

MATERIALS AND METHODS

The study was conducted in the Transcarpathian Territorial Plant Quarantine Centre of the Plant Protection Institute of the National Academy of Agrarian Sciences of Ukraine in 2014-2015. The research was continued at the Institute of Electron Physics, National Academy of Sciences of Ukraine in 2021-2022.

Experiments were conducted under laboratory conditions in fumigation chambers (with a capacity of 30 litres). For this purpose, devices for measuring the concentration of fumigants were used – a PhD-Lite gas analyser, ШІ-11 interferometer, developed device (Mamontov & Romanko, 2010) for obtaining and dosing gases, measuring high concentrations of phosphine, and other necessary laboratory equipment. A thermostat was also used for fumigation at high temperatures.

When conducting laboratory tests of a mixture of phosphine and carbon dioxide, the developed neutral gas dispenser was used, which provided the required dosage in the range from 200 to 2500 ml. For conducting research, the preparative form of phosphine "Magtoxin" (tablet form), carbon dioxide in cylinders was used. Biomaterial for experiments was diluted in the laboratory.

The objects of the study were: the efficiency and synergy of mixtures of phosphine and carbon dioxide gases, the bean weevil at the imago stage (*Acanthoscelides obtectus*), as a biologically close species of quarantine pests absent on the territory of Ukraine to *C. chinensis* and *C. maculatus*.

The effectiveness of phosphine in a mixture with carbon dioxide was determined by the Abbott formula:

$$C\% = \frac{(P_k - P_0)}{P_k} * 100 \qquad (1)$$

where: *C%* – pest death, %; R_k – pest death in control, %; R_o – pest mortality in the experiment,%.

The efficiency of gas mixtures was determined based on the indicator of the product of concentration by time (hereinafter referred to as PCT), which is expressed in units of hours.

The studies aimed to determine the effectiveness of phosphine gases with carbon dioxide against pests of leguminous products under various fumigation parameters: temperature conditions from 21 to 32°C, exposures from 2 to 20 hours and phosphine concentrations from 142 ppm (0.20 g/m^3) up to 702 ppm (1.03 g/m^3), carbon dioxide concentrations from 2.6 to 10.2% (i.e. 52.69 to 204.59 g/m^3), $PCT_{\text{by phosphine}}$ in the range of 0.95-8.08 h*g. Statistical data processing was performed using computer mathematical functions built into Microsoft Excel 2003. Studies with mixtures of gases (phosphine with carbon dioxide) were conducted at the sublethal level of poisoning to identify gas synergy or lack thereof.

The examination of the toxic effect of a mixture of phosphine and carbon dioxide in their various concentrations and exposures during the fumigation of leguminous products against pests was conducted in the following sequence:

A) First, the optimal concentrations of phosphine and carbon dioxide were determined, which would provide the highest synergy of gases. Several variants of the experiment were conducted to do this. Variants of each experiment were: *the examined mixture of gases* (phosphine with carbon dioxide), *standard* (separate action of phosphine), and *control* (non-fumigated pests that were kept at the same temperatures as fumigated ones). After fumigation was completed, species were recorded and average values of insect death were established. The value of the gas synergy indicator was established when determining the difference in insect death between *a mixture of gases* and *standard*.

Various concentrations were tested to determine the optimal concentrations of phosphine and carbon dioxide, which provided the highest synergy of gases, in particular, phosphine 0.2-0.36 g/m^3, 0.54-0.65 g/m^3 and 0.81-1.03 g/m^3, in variations with carbon dioxide 55-60 g/m^3, 110-120 g/m^3, and 200-210 g/m^3, provided that other fumigation parameters, such as temperature and exposure, are the same (Romanko & Dudynska, 2023).

B) After establishing the optimal concentrations of phosphine and carbon dioxide in the mixture, experiments were conducted to determine the effect of the duration of exposures on the synergy index of a mixture of phosphine and carbon dioxide during the fumigation of leguminous products against pests. For this purpose, at the already established optimal concentrations of phosphine and carbon dioxide, experiments were conducted at various exposures (2, 4, 6 and 20 hours). As in previous experiments, the value of the gas synergy indicator was established when determining the difference in insect death between *a mixture of gases* and the *standard*.

C) After establishing optimal concentrations of phosphine and carbon dioxide in the mixture and exposures, experiments were also conducted to determine the effect of temperatures on the synergy index of a mixture of phosphine and carbon dioxide during fumigation against pests. For this purpose, at the already established optimal concentrations of phosphine, carbon dioxide and duration of exposures, four variants of experiments were tested at different temperatures (21-22, 24, 28, 30 and 32°C), provided that other fumigation parameters, in particular, gas concentrations and exposure between the variants of experiments, are the same. As in previous experiments, the value of the gas synergy indicator was established when determining the difference in insect death between *a mixture of gases* and the *standard*.

RESULTS AND DISCUSSION

The obtained research results showed the influence of phosphine and carbon dioxide concentrations on gas synergy in the mixture against the pest at the imago stage. Thus, in experiments with mixtures of phosphine gases (average concentration of 0.81 g/m^3) and carbon dioxide (52.69 g/m^3), an increase in the death rate of adult pests was observed by 3.0±0.57%, compared to the standard (namely, gas synergy) with similar fumigation parameters (temperature 21-22°C, exposure for 6 hours, phosphine concentration in the range of 0.81-0.82 g/m^3, $PCT_{by\ phosphine}$ within 4.85-4.93 h*g) (Fig. 1).

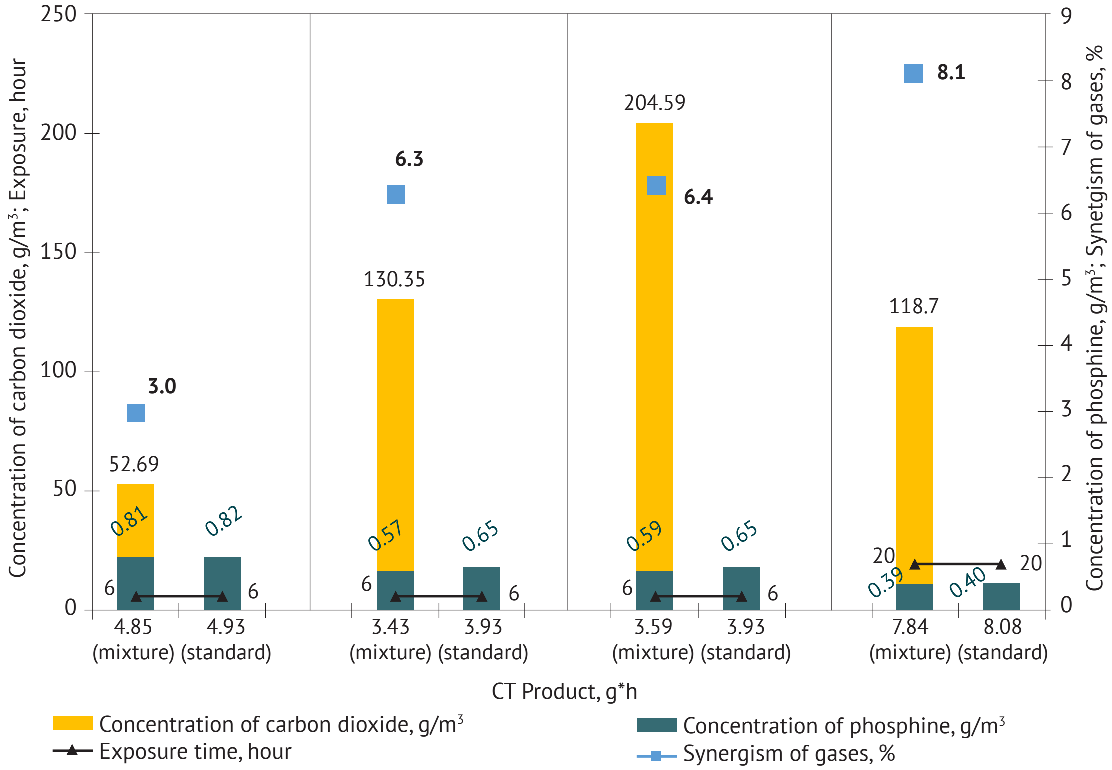

Figure 1. *Dependence of gas synergy on its concentrations and duration of exposures during fumigation against imago pests at a temperature of 21-22°C (laboratory experiments, 2014-2015, 2021-2022)*
Source: *compiled by the authors*

An increase in the concentration of carbon dioxide, even if lower concentrations of phosphine were used, contributed to an increase in the gas synergy index. Thus, in experiments with mixtures of phosphine gases (average concentration 0.57 g/m^3) and carbon dioxide (130.35 g/m^3), an increase in the pest death rate was observed by 6.3±0.43%, compared to the standard for similar fumigation parameters (temperature 21-22°C, exposure 6 hours, phosphine concentration in the range of 0.57-0.65 g/m^3, $PCT_{by\ phosphine}$ in the range of 3.43-3.93 h*g). That is, in comparison with the previous experiment, an increase in the concentration of carbon dioxide by 2.47 times, even if lower concentrations of phosphine are used from 0.81 to 0.57 g/m^3, contributed to a 2.1-fold increase in gas synergy (namely, by 3.3%) (Fig. 1).

However, a further increase in carbon dioxide concentrations above 130.35 g/m^3 did not considerably affect the synergy of gases (with the same fumigation parameters: temperature 21-22°C, exposure 6 hours). Thus, in experiments with mixtures of phosphine gases (average concentration 0.59 g/m^3) and carbon dioxide (204.59 g/m^3) a higher pest death rate of 6.4±0.29% was observed, compared to the standard for similar fumigation parameters (temperature 21-22°C, exposure of 6 hours, phosphine concentration in the range of 0.59-0.65 g/m^3, $PCT_{by\ phosphine}$ in the range of 3.93-3.59 h*g). That is, a further increase in the concentration of carbon dioxide by 1.57 times almost did not contribute to an increase in gas synergy, compared to the previous experiment, where the concentration of carbon dioxide was 130.35 g/m^3.

The results of studies showed that the toxicity of gas mixtures was mainly affected by the duration of exposure, even when using phosphine concentrations below 0.59 g/m^3. Thus, in experiments with mixtures of phosphine gases (average concentration of 0.39 g/m^3) and carbon dioxide (118.70 g/m^3) an increase in the pest death rate by 8.1±0.83% was observed, compared to the standard for similar fumigation parameters (temperature 21-22°C, exposure of 20 hours, phosphine concentration in the range of 0.39-0.40 g/m³, $PCT_{by\ phosphine}$ in the range of 7.84-8.08 h*g). Therefore, in comparison with the previous experiment, increasing the duration of fumigation from 6 hours to 20 not only reduced the concentration of phosphine from 0.59 to 0.39 g/m³ and carbon dioxide from 204.59 to 118.70 g/m³, but also increased the synergy index from 6.4±0.29 to 8.1±0.83% (1.27 times) (Fig. 1).

Thus, the average phosphine concentrations in the range of 0.39-0.82 g/m³ were established and carbon dioxide 52.69-130.35 g/m³, which provide a synergy of gas mixtures in the range of 3.0-8.1% against pests at the imago stage at a temperature of 21-22°C and exposure within 6-20 hours. Subsequent studies have shown the possibility of detecting gas synergy even at phosphine concentrations below 0.39 g/m³, however, at high temperatures (Fig. 2).

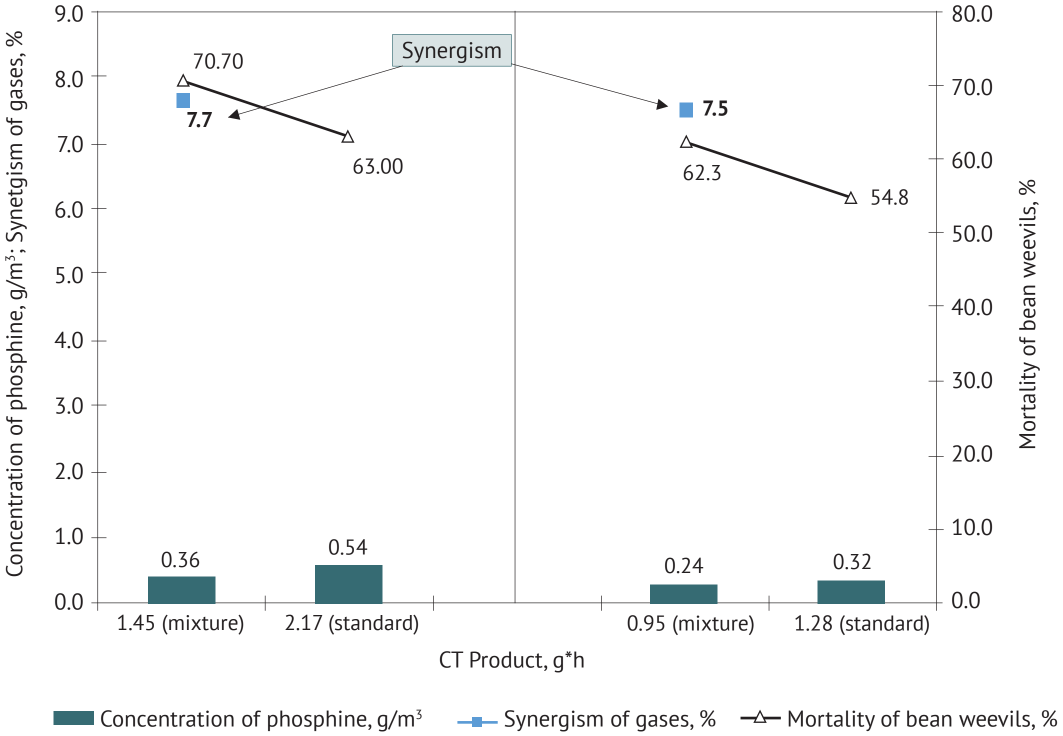

***Figure 2.** A synergy of gases in the mixture during fumigation against imago pests at a temperature of 30°C (laboratory experiments, 2014-2015, 2021-2022)*
***Note**: the concentration of carbon dioxide in the mixture is 116.48 g/m³*
***Source:** compiled by the authors*

Thus, in experiments with mixtures of phosphine gases (average concentration of 0.36 g/m³) and carbon dioxide (116.48 g/m³), an increase in the pest death rate by 7.7±0.53% was observed, compared with the standard for similar fumigation parameters (temperature 30°C, exposure of 4 hours, phosphine concentration 0.54 g/m³, PCT of the standard 2.17 h*g). Further reduction of the phosphine concentration in the gas mixture to 0.24 g/m³ (1.5 times) did not substantially reduce (only by 0.2%) the synergy of gases during fumigation against the pest at the imago stage (Fig. 2).

It was established that with an increase in the duration of fumigation, not only the effectiveness of phosphine (standard) against the imago pest increased, but also the efficiency of gas mixtures. Thus, for an exposure of 2 hours and a temperature of 30°C, $PCT_{by\ phosphine}$ in a mixture of 1.28 h*g; $PCT_{by\ phosphine}$ in the standard of 2.06 h*g, the death of the imago pest was observed only at the level of 35.80±1.98% (in the standard) and 40.20±2.61% (in the mixture). An increase in the duration of fumigation to 4 hours at similar PCT and the same temperature led to an increase in pest death to 63.0±3.64% (in the standard) and 70.7±3.90% (in a mixture of gases). A further increase in exposure to 6 hours contributed to an even higher efficiency of both phosphine and its mixture with carbon dioxide, where their efficiency with similar fumigation parameters was 79.8±3.07% and 90.1±4.13%, respectively.

A tendency to influence the duration of fumigation on the indicator of the synergy of mixtures of phosphine and carbon dioxide gases against pests at the imago stage was also observed. Thus, for an exposure of 2 hours and a temperature of 30°C, an increase in gas synergy in the gas mixture was observed by 4.4±0.66%

compared to the standard ($PCT_{by\ phosphine}$ in a mixture of 1.28 h*g; $PCT_{by\ phosphine}$ in the standard of 2.06 h*g). While at an exposure of 4 hours and a temperature of 30°C, an increase in gas synergy was observed at the level of 7.7±1.61%, compared to the standard ($PCT_{by\ phosphine}$ in a mixture of 1.45 h*g; $PCT_{by\ phosphine}$ in the standard of 2.17 h*g). That is, an increase in the duration of fumigation from 2 to 4 hours led to an increase in gas synergy by 3.3% (from 4.4±0.66 to 7.7±1.61%). A further increase in the duration of fumigation to 6 hours also led to an increase in the gas synergy index by 2.6% (from 7.7±1.61 to 10.3±1.08%) for similar fumigation parameters (temperature 30°C; $PCT_{by\ phosphine}$ in the mixture in the range of 1.25-1.45 h*g; $PCT_{by\ phosphine}$ in the standard of 2.01-2.17 h*g) (Fig. 3).

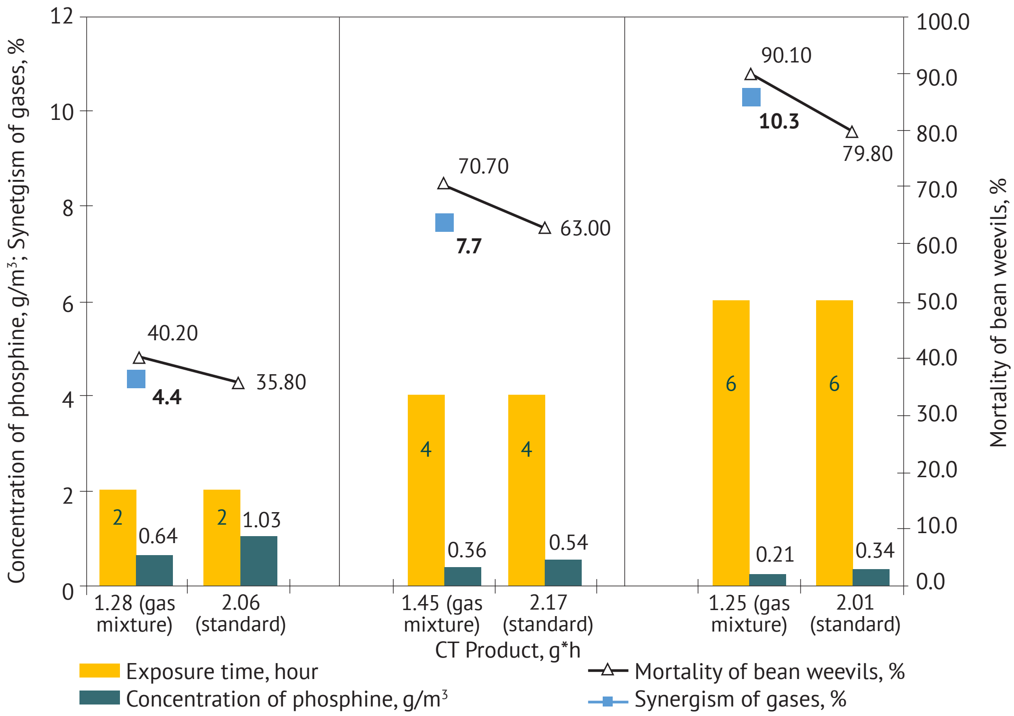

Figure 3. *Dependence of gas synergy on the duration of exposure during fumigation against imago pests at a temperature of 30°C (laboratory experiments, 2014-2015, 2021-2022)*
Note: *the concentration of carbon dioxide in the mixture is 116.48 g/m³*
Source: *compiled by the authors*

Thus, according to the results obtained, at a temperature of 30°C, an increase in gas synergy in the mixture was observed by 4.4±0.66, 7.7±1.61 and 10.3±1.08% compared to the standard for exposure of 2, 4 and 6 hours, respectively (PCT for phosphine in the mixture in the range of 1.25-1.47 h*g; PCT for phosphine in the standard in the range of 2.01-2.17 h*g).

A substantial increase in the efficiency of phosphine and its mixture with carbon dioxide depends on the temperature. Thus, at a temperature of 24°C, the death rate of an imago pest in the standard was 24.9±0.92%, and in a mixture of gases – 28.4±1.02 %. At a temperature of 28°C, the efficiency of the standard was at the level of 44.9±0.41%, and the gas mixture – 50.5±1.03%. At a temperature of 30°C, even greater efficiency was observed in the standard of 63.0±3.67 % (standard) and 70.7±3.90% (mixture of gases). The highest efficiency of phosphine and its mixture (up to 78.7±1.14 %) with carbon dioxide was observed at a temperature of 32°C.

Notably, the obtained results confirmed the influence of temperature on the synergy of mixtures of phosphine gases with carbon dioxide against pests at the imago stage. Therefore, when the temperature increased, an increase in the synergy of gases in the mixture against the pest at the imago stage was observed.

Thus, a temperature growth from 24 to 28°C led to an increase in the synergy index of gas mixtures from 3.5±0.40 to 5.6±0.73% for similar fumigation parameters (exposure of 4 hours, phosphine concentration in the standard in the range of 0.53-0.56 g/m³; a mixture of 0.32-0.35 g/m³; carbon dioxide concentration of 116.48 g/m³; for *standard* $PCT_{by\ phosphine}$ within 2.12-2.23 h*g, for *mixtures* $PCT_{by\ phosphine}$ within 1.28-1.39 h*g) (Fig. 4). A further increase in temperature by two degrees (from 28 to 30°C) led to an increase in the synergy index of gas mixtures by 2.1% (from 5.6±0.73 to 7.7±1.61% with similar fumigation parameters (exposure of 4 hours, phosphine concentration in the standard in the range of 0.53-0.54 g/m³; a mixture of 0.35-0.36 g/m³; carbon dioxide concentration 116.48 g/m³; for *standard* $PCT_{by\ phosphine}$ within 2.12-2.17 h*g, for *mixtures* $PCT_{by\ phosphine}$ within 1.39-1.45 h*g).

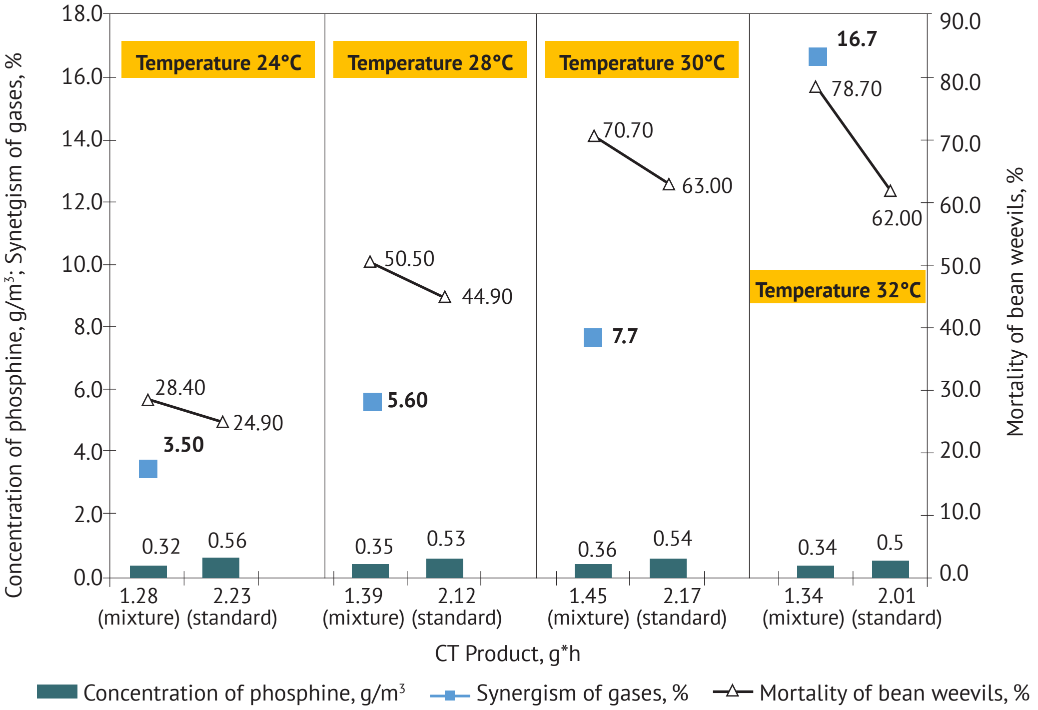

Figure 4. *Dependence of gas synergy on temperature during fumigation against imago pests (laboratory experiments, 2014-2015, 2021-2022)*
Note: *the concentration of carbon dioxide in the mixture is 116.48 g/m^3, exposure time – 4 hours*
Source: *compiled by the authors*

The highest synergy of gas mixtures (16.7±0.52%) was observed at a temperature of 32°C with the following parameters: exposure of 4 hours, average phosphine concentration 0.34 g/m^3, carbon dioxide 116.48 g/m^3 for *mixtures* $PCT_{by\ phosphine}$ 1.34 h*g. While the average phosphine concentration in the standard was 0.5 g/m^3, and the PCT is 2.01 h*g. Notably, the increase in temperature from 30 to 32°C led to an increase in the gas synergy index by 9%, that is, by 2.17 times.

Thus, at temperatures of 24, 28, 30, and 32°C, there was an increase by 3.5±0.40, 5.6±0.73, 7.7±1.61 and 16.7±0.52%, respectively, of imago *A. obtectus* mortality under the action of mixtures of phosphine with carbon dioxide in comparison with the standard. Fumigation at a temperature of 30-32°C allowed to use low concentrations of phosphine, in the range of 0.21-0.36 g/m^3, which contributed to a reduction in phosphine costs by 33.3-38.6% compared to the standard.

Notably, the indicator of gas synergy can be affected not only by fumigation parameters but also by the variety of pests and disinfection methods. Thus, Klechkovsky & Neamtsu (2019) stated that a mixture of methyl bromide and carbon dioxide can provide 100% death of *Frankliniella occidentalis* Perg, reducing the dosage of fumigant by 1.6-2 times.

Whereas, Neamtsu (2018) claims that when using carbon dioxide in mixtures with methyl bromide, the lethal rate (product of concentration by exposure time) against *Aleyrodes proletella* can decrease even by 3-3.8 times. Therewith, the efficiency of 100% fumigation was observed. The author claimed that the above result was achieved due to an increase in temperature to 31°C. This is due to the fact that the heated gaseous mixture of carbon dioxide and a toxic agent (fumigant) is capable of their rapid and thorough physical mixing, as well as due to the increase in the diffusion process tenfold, active transportation to the respiratory system of the pest.

Jagadeesan *et al.* (2018) proved that fumigation with mixtures of phosphine and sulfuryl fluoride did not lead to resistance of such major grain insect pests as *Sitophilus oryzae* and *Cryptolestes ferrugineus*. In addition, the researchers determined the synergy of the mixtures, compared not only with the individual action of phosphine but also with sulfuryl fluoride. Thus, at a temperature of 25°C, fumigation with mixtures PH_3 with SO_2F_2 led to a decrease in the concentration of phosphine in the range of 2.5-4.2 times, depending on the type of pest. For example, the use of a mixture of fumigants allowed to reduce phosphine consumption from 14.2-14.5 to 5.6-6.36 mg/l and from 2.71-5.03 to 0.93-1.2 mg/l respectively, for *C. ferrugineus* and *S. oryzae*. Therewith, a high (99.9%) fumigation efficiency was observed.

Based on the above data, the synergy indicator is influenced not only by the selection of fumigation parameters but also by the variety of pests.

Jagadeesan *et al.* (2021) confirmed that the synergy of mixtures of sulfuryl fluoride and phosphine can be achieved in two ways: their joint action during continuous 168 hours (PH_3+SO_2F_2) and sequentially for two periods of 78 hours, during which the insects were first exposed to sulfuryl fluoride and then phosphine with 12-hour aeration ($SO_2F_2 \rightarrow PH_3$). Continuous application of gas mixtures for two fumigation parameters as for sulfuryl fluoride 185 + phosphine 168 g hm^{-3}, and as for SO_2F_2 370+PH_3 84 g hm^{-3}, provided full control over imago and eggs of *Cryptolestes ferrugineus*. The above disinfection parameters were also efficient with the consistent use of fumigants. Notably, regardless of the methods of application, individual actions of the examined fumigants did not ensure the complete death of the pest, even at the imago stage.

Manivannan *et al.* (2016) examined various populations of phosphine-resistant *Rhyzopertha dominica* at different concentrations of fumigant and its mixtures with 10, 20, and 30% carbon dioxide at diverse time intervals at 25°C. Gas synergy was detected at all proposed exposures (lasting 4, 6, and 7 days) compared to a single phosphine action. Adding 30% carbon dioxide to low phosphine concentrations and a 4-day exposure time provided a better synergistic effect during fumigation. Studies have shown that carbon dioxide enhances the toxic effects of phosphine, thereby reducing the concentration and exposure time during fumigation against different populations of *R. dominica*.

Constantin *et al.* (2020) noted that fumigation of phosphine with carbon dioxide against *Cryptolestes ferrugineus* provides 2.8-fold savings in fumigant (the fumigant concentration was reduced from 16.2 to 5.8 mg/litre). A comparison of pest death data from a separate phosphine action (standard) and a pH mixture$_3$+CO_2 showed that carbon dioxide increases phosphine toxicity. These results were confirmed by three independently obtained populations of *C. ferrugineus* in field conditions, which differed in resistance. Studies have confirmed the synergy of gas mixtures observed in all the examined pest populations, including highly resistant ones. Thus, this study is consistent with the literature data, primarily on the effect of various fumigation parameters (temperature, duration of exposure, and gas concentration) on gas synergy.

CONCLUSIONS

The synergism and toxic effect of a mixture of phosphine and carbon dioxide against pests was examined under various fumigation parameters. Optimal concentrations of phosphine in the mixture at which synergy was observed during fumigation against adult pests were established: at low temperatures (21-22°C) – in the range of 0.57-0.82 g/m^3; at high temperatures (31-32°C) – in the range of 0.21-0.36 g/m^3.

The optimal concentration of carbon dioxide, at which its substantial effectiveness was shown, was in the range of 110-130 g/m^3 (or 5.5-6.5% of the total air volume), regardless of the temperature indicator. The effect of fumigation duration on the efficiency and synergy of gases in the mixture was observed. Namely, there was a clear trend: with an increase in the duration of fumigation, both the efficiency of action and the indicator of the synergy of gas mixtures increased. The highest synergism in fumigation against imago pests was 10.3±1.08%.

The temperature was the most substantial factor influencing the value of the gas synergy index in a mixture of phosphine and carbon dioxide. The highest rates of the synergy of gas mixtures were noted precisely at high fumigation temperatures (32°C) against imago pests (16.7±0.52%).

The use of high temperatures and relatively long exposures allowed using low concentrations of phosphine in a mixture of gases in the range of 0.21-036 g/m^3, and, as a result, this contributed not only to reducing the cost of phosphine to 38.6% compared to the standard, but it also did not affect the decrease in the value of the synergy indicator of gas mixtures.

The results obtained indicate the need for further research, in particular, the investigation of the toxic effect of gas mixtures against different populations of pests at their various stages of development. It is also vital to establish the possibility of obtaining 100% pest death, including at the pupal and egg stages with the prospect of using mixtures of phosphine and carbon dioxide and in quarantine fumigation.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Синергізм суміші фосфіну та вуглекислого газу при фумігації проти зерноїдів

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Анотація. Робота присвячена пошуку альтернатив бромистому метилу – універсального фуміганта, який був обмежений у застосуванні на вимогу Монреальського протоколу. Мета: визначити синергізм суміші фосфіну та вуглекислого газу у різних їх концентраціях та експозиціях при фумігації зернобобової продукції проти зерноїдів за високих температур. Методи: аналітичний огляд з тематики досліджень, чинній нормативно-правовій базі у галузі знезараження; аналіз біологічних особливостей комах-шкідників зернобобової продукції; експериментальний – варіювання різних концентрацій фосфіну, тривалості експозиції та температури для встановлення синергізму фуміганта із вуглекислим газом у лабораторних умовах за відповідного обладнання; математико-статистичний – за допомогою комп'ютерних математичних функцій, вбудованих у програму Microsoft Excel 2003. При фумігації сумішей газів проти зерноїдів на стадії імаго оптимальна концентрація вуглекислого газу знаходиться в межах 110-130 г/м^3 (або 5,5-6,5 % від загального об'єму повітря) незалежно від температурного показника. Тоді як оптимальна концентрація фосфіну за невисоких температур (21-22 °C) – в межах 0,57-0,82 г/м^3, а при високих (31-32 °C) – в діапазоні 0,21-0,36 г/м^3. При збільшенні тривалості фумігації підвищувалася не лише ефективність фосфіну (еталону) проти імаго зерноїда, а також і ефективність сумішей газів. Крім того виявили залежність синергізму газів від тривалості фумігації. Так, за температури 30 °C спостерігали підвищення синергізму газів у суміші на 4,4±0,66, 7,7±1,61 та 10,3±1,08 % порівняно з еталоном за експозиції 2, 4 та 6 годин відповідно. Температура виявилася найбільш визначальним фактором, що впливав на значення показника синергізму газів у суміші фосфіну з вуглекислим газом. Так, підвищення температури з 30 до 32 °C сприяло збільшенню показника синергізму газів на 9 %, тобто у 2,17 разів. Подальше встановлення летальних норм та режимів фумігації проти зерноїдів в тому числі і карантинних дозволяють запобігти економічних збитків від даних шкідників в Україні і будуть сприяти виконанню рішень Монреальського протоколу, спрямованого на охорону довкілля і запобіганню руйнування озонового шару

Ключові слова: альтернатива бромистому метилу; концентрація; тривалість експозиції; температура; зерноїди