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Effectiveness of weed control by tank mixture of herbicides aclonifen and prometryn on sunflower crops

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Abstract

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This work is devoted to the elaboration of herbicides composition that would provide effective protection of sunflower (*Helianthus annuus* L.) crops from weeds, as well as it would be an effective means of preventing emergence of herbicide-resistant weed biotypes. For this purpose, the interaction effects, weed control efficiency, and crop selectivity had been studied in greenhouse experiment and field trial with tank mixtures of herbicide inhibitor of carotenoid synthesis aclonifen and herbicide inhibitor of electron transport in the photosystem 2 of chloroplasts prometryn. The herbicides aclonifen and prometryn were applied at different application rates separately and in the mixtures to the soil before the emergence of plant seedlings. It has been shown that in the mixtures of herbicides aclonifen and prometryn the interaction is mainly additive. The mixture of herbicides is selective for sunflower in the range of application rates of aclonifen 1.8–2.4 kg ha^{-1} and of prometryn 1–1.5 kg ha^{-1}. High efficiency of weed control by the mixture was achieved with the aclonifen and prometryn application rates, respectively, 1.8 and 1.5 kg ha^{-1}, or 2.4 and 1.0 kg ha^{-1}. The effectiveness of weed control in field conditions with mixtures of herbicides aclonifen and promethrin at the specified rate ratio, was not inferior to the complex herbicide Primekstra TZ Gold at the recommended application rate of 4.5 L ha^{-1}.

Keywords: Helianthus annuus L.; tank mixture; resistance; interaction; aclonifen; prometryn

Introduction

The emergence and spread of herbicide-resistant weed biotypes due to the selection pressure, which exerted by the permanent use of herbicides is one of the central problems of the modern chemical method of crop protection (Vencill et al., 2012; Kraehmer et al., 2014; Gaines et al., 2020; Perotti et al., 2020). Despite the fundamental character of the problem of resistance and uncertainty about the possibility of its radical solution (Shaw, 2016; Barrett et al., 2017; Harker et al., 2017), there is not a doubt that for decreasing the emergence of herbicide-resistant weed biotypes the reduction of narrowly targeted selection pressure of herbicides is important, that can be achieved through the complex use of herbicides with different mode of action (Norsworthy et al., 2012).

For sunflower (*H. annuus* L.) crop protection are widely used tank mixtures of herbicides and complex herbicides that consist of herbicides long-chain fatty acid synthesis (LCFA) inhibitors, which are mainly effective against grass weeds, and herbicides electron transport (ET) inhibitors in the photosystem (PS) 2 of chloroplasts, which are effective mainly against dicotyledonous weeds (Morderer et al., 2014; Rafalsky et al., 2018). In this case, the main goal of complex herbicide application is to increase the effectiveness of crop protection by expanding the range of controlled weed species. Therefore, the active ingredients of herbicides that complement each other in the spectrum of action are selected for complex use. However, to prevent the emergence of resistance by reducing the direction of herbicides selection pressure, it is necessary to choose the components of herbicide compositions with similar spectrum of action (Norsworthy et al., 2012). Under such conditions, high protection efficiency can be achieved only with the additive or synergistic interaction of herbicide mixture components. An obvious requirement for the components of herbicides mixture is their selectivity for crop.

Given the above requirements, the anti-resistant mixture for the protection of sunflower crops may include the herbicide aclonifen, which is the carotenoid synthesis inhibitor and herbicide prometryn, which is an ET inhibitor in PS 2. Arguments in favor of such composition of the anti-resistant mixture are as follows. – firstly, the herbicides aclonifen and prometryn are recommended for use in sunflower crops (Rafalsky et al., 2018) and therefore, are selective for this crop, while the spectra of action of aclonifen and prometryn significantly intersect (Schwartau, 2009). Secondly, no aclonifen-resistant weed biotype has been recorded to date (Heap, 2021). There is also a reason to expect that the interaction of aclonifen with prometryn may be synergistic. According to its chemical structure, aclonifen belongs to diphenyl ethers, which are inhibitors of the enzyme protoporphyrinogen oxidase (PPO). Despite its structural similarity to PPO inhibitors, the phytotoxic effect of aclonifen develops in a completely different way, and is characterized by a bleaching effect (leaf whitening), which is peculiar to herbicides that inhibit carotenoid biosynthesis. The site of action of aclonifen has been found to be the enzyme Solanesyl diphosphate synthase (SPS), blocking the activity of which inhibits carotenoid biosynthesis (Kahlau et al., 2020). It is well known that herbicides inhibitors of carotenoid biosynthesis from the class of hydroxyphenylpyruvate dioxygenase (HPPD) inhibitors synergistically interact with herbicides inhibitors of ET in PS 2 of chloroplasts (Armel et al., 2008; Walsh et al., 2012; O'Brien et al., 2018; Osipitan et al., 2018; Willemse et al., 2021). Because aclonifen is an inhibitor of carotenoid biosynthesis, although with a different mechanism of action than HPPD inhibition, synergism can be expected, when it jointly applied with ET inhibitors.

Nevertheless, given the structural similarity of aclonifen with herbicides PPO inhibitors, the possibility of antagonism in the mixtures with herbicides ET inhibitors cannot be ruled out. Thus, antagonistic interaction was observed, when the herbicide PPO inhibitor flumioxazine was applied to the soil before the emergence of sunflower seedlings in a mixture with the herbicide inhibitor ET prometryn (Radchenko et al., 2022). Antagonism was also observed, when the ET inhibitor metribuzin was jointly applied with PPO inhibitor carfentrazone on plant seedlings (Yukhymuk et al., 2021).

The aim of this study is to determine the character of the interaction, the effectiveness of weed control and crop selectivity when herbicides aclonifen and prometryn jointly, applied in sunflower crops in order to create tank mixture that is effective in protecting sunflower crops.

Materials and Methods

The initial assessment of effective application rates and interaction effects at the use of tank mixtures was performed in greenhouse experiment on model objects. Cultivated oilseed radish plants (*Raphanus sativus* d. var. *oleifera* Metrg.) were used as a model of annual dicotyledonous weeds, and sowing oat (*Avena sativa* L.) as a model of annual grass weeds. Plants were grown in plastic containers with a capacity of 1.3 kg of soil on the vegetation site of the Institute of Plant Physiology and Genetics of National Academy of Sciences of Ukraine. For each variant of the mixture, 8 pots with plants were grown: 4 pots oat sowing and 4 pots with oilseed radish. 20 plants grew in each pot with sowing oat, 15 plants were grown in pots with oilseed radish. The greenhouse experiment was repeated two times.

Herbicides aclonifen and prometryn were applied separately and in mixtures, within the recommended range of application rates: for aclonifen 0.6, 1.2, 1.8 and 2.4 kg ha^{-1}, for prometryn – 1.0 and 1.5 kg ha^{-1}. The pot was filled with substrate almost completely, seeds were sown. The amount of herbicide measured per the area of the pots was mixed with the soil, and then the sown seeds were covered with it in a layer of 1 cm.

The phytotoxic effect of herbicides was evaluated by inhibiting dry matter accumulation of the aboveground part and the content of photosynthetic pigments in the leaves of plants. To determine photosynthetic pigments content, (Welburn, 1994) leaves were taken from several plants and crushed. 0.05 mg of material was taken and placed in test tubes with 5 ml of dimethyl sulfoxide. Samples of plant material were extracted in dimethyl sulfoxide in a water bath at 67°C for 3 h; then 1 ml was taken from each test tube and added to 4 ml of dimethyl sulfoxide. The resulting solution was analyzed spectrophotometrically, pigments contents were calculated per unit mass of dry matter and analysis was performed in four replicates.

The effectiveness of weed control and the selectivity of herbicide tank mixture for sunflower were determined in field experiment. Field experiment in crops of sunflower hybrid 'Neoma' was conducted in 2020–2021, in the fields of the research farm of the Institute of Plant Physiology and Genetics of National Academy of Sciences of Ukraine, Glevakha village (50°16´ N, 30°18´ E), Fastiv district, Kyiv region. The predecessor was winter wheat. The research farm is located on the border of Polissia and Forest-Steppe zones. The climate is temperate, the annual rainfall is from 520 to 645 mm. Sod-podzolic soil, loamy in mechanical composition, humus content 1.6%, pH 5.6.

Herbicides were applied by spraying the soil after sowing, but before the emergence of sunflower seedlings, using a backpack rod sprayer with compressed air: rodlenght 3 m, number of nozzles 6, distance between nozzles 50 cm, distance to the target 50 cm, speed 5 km h^{-1}, working fluid consumption 300 L ha^{-1}.

The experiment was arranged using a randomized complete block design with four replicates. The area of the experimental plot was 15 m^2 (3 × 5 m).

The effectiveness of weed control was determined for each species separately by counting the number of weeds in areas with herbicide application, compared to the control and was calculated by the formula (Ivashchenko, Merezhinsky, 2001):

$$E (\%) = 100 – K_2 \cdot 100/K_1), \quad (1)$$

where E (%) – is the controlling efficiency of a certain weed species; K_1- number of weeds of a certain species per 1 m^2 in control; K_2- the number of weeds of a certain species per 1 m^2 on the treated area. Weed accountings were performed 24 and 56 days after herbicide application, as well as before sunflower seed harvesting.

Interaction effects when applying a mixture of herbicides were determined by the method of Colby (1967), by comparing the actual inhibitory effect or effectiveness of controlling of a certain weed species observed when using this mixture, with the expected one calculated by formula:

$$E_{12} = E_1 + E_2 \cdot (100 – E_1)/100. \quad (2)$$

where E_{12} – is the expected inhibitory effect or effectiveness of weed controlling under the action of the herbicide mixture; E_1 and E_2 are inhibitory effect or effectiveness of weed controlling by the first and second components of the mixture respectively.

The selectivity of herbicides for crop was assessed by conducting biometric measurements and phenological observations, according to standard methods (Ivashchenko, Merezhinsky, 2001).

Sunflower seed yield accounting was performed by harvesting and threshing heads from the accounting plots. Seeds were weighted and moisture was determined. The yield was calculated in t ha^{-1} at a standard humidity of 8%.

In the experiment, the following herbicides were used: Challenge 600 SC (a.i. aclonifen, 600 g L^{-1}), Gesagard 500 FW, SC (a.i. prometryn, 500 g L^{-1}). For comparison, the complex herbicide Primekstra TZ Gold was used at the rate of 4.5 L ha^{-1} (S-metolachlor, 1.4 kg ha^{-1} + terbuthylazine, 0.84 kg ha^{-1}). The scheme of field experiment is given in Table 1.

Table 1. Scheme of field experiment

No.	Treatment
1	Control
2	Aclonifen (1.8 kg ha^{-1})
3	Aclonifen (2.4 kg ha^{-1})
4	Prometryn (1.0 kg ha^{-1})
5	Prometryn (1.5 kg ha^{-1})
6	Aclonifen (1.8 kg ha^{-1}) + Prometryn (1.0 kg ha^{-1})
7	Aclonifen (1.8 kg ha^{-1}) + Prometryn (1.5 kg ha^{-1})
8	Aclonifen (2.4 kg ha^{-1}) + Prometryn (1.0 kg ha^{-1})
9	Aclonifen (2.4 kg ha^{-1}) + Prometryn (1.5 kg ha^{-1})
10	S-metolachlor (1.4 kg ha^{-1}) + terbuthylazine (0.84 kg ha^{-1})

Note: control – herbicides were not applied.

The treatment by herbicides in field experiment in 2020 was carried out on 27 April, one day after sowing sunflowers. At the time of treatment was cloudy weather (80%), air temperature 12°C, wind 5 m/s, moist soil. In 2021, sowing of sunflower was carried out at a later date and, respectively, the application of herbicides was carried out on 6 May, one day after sowing. At the time of treatment, there was cloudy weather (90%), air temperature 13°C, wind 4 m s^{-1}, soil surface dry, moisture was at a depth of 3 cm.

Statistical analysis of the results was performed by analysis of variance (ANOVA), using the Tukey (HSR) test. The results were presented as mean and standard errors (m ± SE). Differences between data were considered significant at $P \leq 0.05$.

Results and Discussion

Greenhouse experiment

In the greenhouse experiment, the visible symptoms of oilseed radish plants injure by herbicides were observed on the 8th day after seedlings emergence. Significant inhibition of dry matter accumulation by radish plants, occurred in the treatments with the applications of the aclonifen separately in the rates of 1.8 and 2.4 kg ha^{-1} and its mixtures with prometryn (Table 2).

When using prometryn separately at both rates of application, there was a tendency to inhibit the accumulation of plant biomass, but the difference with control was not significant. The addition of prometryn to aclonifen provided

Table 2. Contents of dry matter mass (DMW), chlorophyll (C_a+C_b) and carotenoids (C_{car}) in the leaves of oilseed radish on the 8th day after the emergence of seedlings

No.	Treatment	DMW	C_a+C_b	C_{car}
1	Control	27.7 ± 1.2a	3.49 ± 0.16ab	0.59 ± 0.02bc
2	Aclonifen (0.6 kg ha^{-1})	25.0 ± 1.4a	3.32 ± 0.19ab	0.63 ± 0.03b
3	Aclonifen (1.2 kg ha^{-1})	21.9 ± 1.2ab	3.30 ± 0.18ab	0.65 ± 0.02b
4	Aclonifen (1.8 kg ha^{-1})	16.5± 1.1bc	3.20 ± 0.20ab	0.66 ± 0.02b
5	Aclonifen (2.4 kg h^{-1})	9.6± 0.7c	2.90 ± 0.29b	0.62 ± 0.02b
6	Prometryn (1.0 kg ha^{-1})	21.4 ± 1.3ab	4.43 ± 0.29a	0.76 ± 0.02a
7	Prometryn (1.5 kg ha^{-1})	21.1 ± 1.8ab	3.66 ± 0.31ab	0.71 ± 0.04ab
8	Aclonifen (0.6 kg ha^{-1}) + Prometryn (1.0 kg ha^{-1})	12.8 ± 1.6c	2.70 ± 0.33bc	0.50 ± 0.01c
9	Aclonifen (0.6 kg ha^{-1}) + Prometryn (1.5 kg ha^{-1})	17.1 ±1.9b	2.73 ± 0.30bc	0.65 ± 0.02b
10	Aclonifen (1.2 kg ha^{-1}) + Prometryn (1.0 kg ha^{-1})	10.7 ± 0.9cd	2.66 ± 0.21bc	0.51 ± 0.01cd
11	Aclonifen (1.2 kg ha^{-1}) + Prometryn (1.5 kg ha^{-1})	10.8 ± 1.4cd	2.46 ± 0.32c	0.49 ± 0.01d
12	Aclonifen (1.8 kg ha^{-1}) + Prometryn (1.0 kg ha^{-1})	10.8 ± 1,2cd	2.26 ± 0.24c	0.46 ± 0.01de
13	Aclonifen (1.8 kg ha^{-1}) +Prometryn (1.5 kg ha^{-1})	8.8 ± 0,5d	1.65 ± 0.12c	0.37 ± 0,01ef
14	Aclonifen (2.4 kg ha^{-1}) + Prometryn (1.0 kg ha^{-1})	8.6 ± 0,6d	2.04 ± 0.13c	0.48 ± 0,01d
15	Aclonifen (2.4 kg ha^{-1}) + Prometryn (1.5 kg ha^{-1})	8.7 ± 0,8d	1.59 ± 0.20c	0.34 ± 0.01f

Note. The average content of dry matter was calculated in mg per 1 plant, the content of photosynthetic pigments is presented in mg g^{-1} of dry matter; differences in averages are significant at $P \leq 0.05$ in the absence of identical letters; comparisons were performed using the Tukey test; comparisons were made within the columns; x ± SE; n = 4; control – wihthout herbicide treatment

almost the same increase in the inhibitory effect on biomass accumulation, at the rates of application of the aclonifen from 0.6 to 1.8 kg ha^{-1} and both rates of application of prometryn. The maximum inhibition of plant biomass accumulation by herbicides mixture was achieved with the application rates of aclonifen 1.8 kg ha^{-1} and prometryn 1.5 kg ha^{-1} or aclonifen 2.4 kg ha^{-1} and prometryn 1.0 kg ha^{-1}. At the rate of application of the aclonifen 2.4 kg ha^{-1}, increasing the rate of application of prometryn to 1.5 kg ha^{-1} did not increase the inhibitory effect. The character of herbicides effect on the accumulation of photosynthetic pigments in the leaves of oilseed radish was not fundamentally different from the effect on the accumulation of plant biomass. The only difference was the tendency to increase the content of chlorophyll and carotenoids under the action of prometryn alone.

The increase in the content of pigments is not an unambiguous sign of the stimulating effect of herbicides. This trend is probably due to the fact, that at the initial stage of the development of phytotoxic action of herbicide inhibitor ET, the inhibition of dry matter accumulation precedes the loss of chlorophyll content. Besides, at low rates of application of herbicides inhibitors, ET can take place the effect "of increasing the green colour" of the leaves, which is due to the fact that the suppression of the photosynthesis products formation by herbicides, ET inhibitors are perceived by the plant as shading, which stimulate the chlorophyll formation (Fedtke, 1985). The effect "of increasing the green colour", can result both in self-sensitilization of plant and increased phytotoxic effects, and vice versa in its reduction. In particular, antagonistic reduction of phytotoxic action of herbicides inhibitors, ET is observed in the mixtures with herbicides derived from dinitroaniline and chloroacetanilide that cause effect "of increasing the green colour" and thus can stimulate chlorophyll accumulation in tolerant plant species (Morderer & Merezhynsky, 2009). Nevertheless, increasing the content of photosynthetic pigments under the action of prometryn alone, did not lead to a decrease in the inhibitory effect of aclonifen and prometryn mixture on radish plants. On the contrary, when adding prometryn at the rate of 1.0 kg ha^{-1}, the inhibitory effect on the accumulation of chlorophyll significantly increased, compared with the effect of the aclonifen alone at the application rates of aclonifen from 1.8 kg ha^{-1}. Addition of higher prometryn application rate (1.5 kg ha^{-1}) to aclonifen leads to significant increase in the inhibitory effect, starting with the lower aclonifen application rate (1.2 kg ha^{-1}).

Sowing oat plants were more resistant to the studied herbicides than oilseed radish plants. Due to this, the phytotoxic effect developed more slowly: if in the radish plants the clear visible symptoms of phytotoxic action in the vast majority of treatments of experiment occurred on the 8th day after emergence, while in oat plants the visible symptoms of phytotoxic action appeared only on the 11th day and only in some treatments of the experiment. When applied separately, aclonifen in the whole range of application rates and prometryn at the rate of 1.0 kg ha^{-1} had almost no effect on the accumulation of dry matter and the contents of photosynthetic pigments in the leaves of oat plants (Table 3).

A significant decrease in the dry matter accumulation of oat plants and the content of photosynthetic pigments, compared to the control was observed, only when applying pro-

Table 3. Contents of dry matter mass (DMW), chlorophyll (C_a+C_b) and carotenoids (C_{car}) in the leaves of sowing oat on the 11th day after the emergence of seedlings

No.	Treatments	DMW	C_a+C_b	C_{car}
1	Control	21.1 ± 1.1a	6.40 ± 0.13a	0.95 ± 0.04a
2	Aclonifen (0.6 kg ha^{-1})	19.1 ± 1.3a	6.05 ± 0,11a	1.04 ± 0,04a
3	Aclonifen (1.2 kg ha^{-1})	20.7 ± 1.3a	6.13 ± 0.10a	1.03 ± 0.01a
4	Aclonifen (1.8 kg ha^{-1})	20.7 ± 1.2a	7.00 ± 0.25a	1.13 ± 0.03a
5	Aclonifen (2.4 kg ha^{-1})	21.7 ± 1.4a	5.82 ± 0.28a	1.04 ± 0.06a
6	Prometryn (1.0 kg ha^{-1})	20.9 ± 1.1a	6.60 ± 0.11a	0.92 ± 0.03a
7	Prometryn (1.5 kg ha^{-1})	10.7 ± 0.7b	3.99 ± 0.21c	0.51 ± 0.01c
8	Aclonifen (0.6 kg ha^{-1}) + Prometryn (1.0 kg ha^{-1})	18.1 ± 0.9a	5.62 ± 0.30a	0.75 ± 0.04b
9	Aclonifen (0.6 kg ha^{-1}) +Prometryn (1.5 kg ha^{-1})	17.9 ± 0.8a	5.42 ± 0.16b	0.73 ± 0.01b
10	Aclonifen (1.2 kg ha^{-1}) + Prometryn (1.0 kg ha^{-1})	19.7 ± 1.0a	5.60 ± 0.12ab	0.96 ± 0.02a
11	Aclonifen (1.2 kg ha^{-1}) + Prometryn (1.5 kg ha^{-1})	16.7 ± 0.6a	5.10 ± 0.09b	1.01 ± 0.04a
12	Aclonifen (1.8 kg ha^{-1}) + Prometryn (1.0 kg ha^{-1})	16.1 ± 0.5a	4.52 ± 0.30c	0.81 ± 0.07ab
13	Aclonifen (1.8 kg ha^{-1}) + Prometryn (1.5 kg ha^{-1})	18.8 ± 0.7a	4.55 ± 0.08bc	0.78 ± 0.01ab
14	Aclonifen (2.4 kg ha^{-1}) + Prometryn (1.0 kg ha^{-1})	14.4 ± 0.4b	4.81 ± 0.13bc	0.78 ± 0.01ab
15	Aclonifen (2.4 kg ha^{-1}) + Prometryn (1.5 kg ha^{-1})	14.3 ± 0.4b	3.95 ± 0.10c	0.69 ± 0.02b

Note: Explanations under Table 2

metryn separately at the rate of 1.5 kg ha^{-1}. Adding aclonifen to prometryn led to an antagonistic reduction in prometryn's effect on carotenoid accumulation. Antagonistic reduction of the phytotoxic effect of the mixture of prometryn and aclonifen on the accumulation of dry matter was observed at the rates of application of aclonifen 0.6-1.8 kg ha^{-1}. The effect of the mixture on the accumulation of plant biomass did not differ significantly from the action of prometryn separately, only at the maximum rate of application of the aclonifen of 2.4 kg ha^{-1}. Antagonistic reduction of prometryn's influence on the accumulation of chlorophyll content, when used in the mixture, was observed in a slightly narrower range of aclonifen application rates. When adding aclonifen, the chlorophyll content did not differ significantly from the treatments with the use of prometryn separately at the rates of application of aclonifen 1.8 and 2.4 L ha^{-1}.

Field experiment

In order to study the effect of the mixture in the field, the aclonifen application rates of 1.8 and 2.4 kg ha^{-1} and prometryn 1.0 and 1.5 kg ha^{-1} were chosen. Weed accountings, conducted on 24 days after herbicide application, when sunflower plants reached the 2-leaf stage, showed that crop clogging had a mixed character in both years. The main threat during both years of research was annual weeds barnyard grass *Echinochloa crus-galli* L. Pal. Beauv. (ECHCG), yellow foxtail *Setaria glauca* L. Pal. Beauv. (SETPF), and annual dicotyledonous weeds shepherd's purse *Capsella bursa-pastoris* L. Medicus (CAPBP), lamb's quarters *Chenopodium album* L. (CHEAL), black bindweed *Polygonum convolvulus* L. (POLCO). In 2020, there were dicotyledonous weeds mustard *Brassica campestris* L. (BRACA), birdweed *Polygonum aviculare* L. (POLAV); in 2021– wild radish *Raphanus raphanistrum* L. (RAPRA), field pansy *Viola arvensis* Murr. (VIOAR). The results of determining the effectiveness of weed control by herbicides are given in Table 4.

Table 4. Effectiveness (%) of weeds control by herbicides in sunflower crops on 24 days after application in 2020 (x ± SE; n = 4)

No.	ECHCG	SETPF	BRACA	CAPBP	CHEAL	POLCO	POLAV
2	53.8 ± 2.1a	47.5 ± 3.5a	99.0 ± 1.1a	99.0 ± 1.5a	72.5 ± 5.3a	37.5 ± 5.2a	27.5 ± 3.4a
3	70.0 ± 7.4b	60.0 ± 4.3b	99.0 ± 1.5a	99.0 ± 1.3a	92.5 ± 3.5c	47.5 ± 5.1ab	35.0 ± 3.3b
4	72.5 ± 5.4b	47.5 ± 3.1a	96.8 ± 2.5a	99.0 ± 1.3a	86.3 ± 6.5b	45.0 ± 6.4a	68.8 ± 3.4c
5	76.3 ± 5.6bc	60.0 ± 2.2b	99.0 ± 1.6a	99.0 ± 1.7a	88.8 ± 6.3b	52.5 ± 3.4b	78.8 ± 1.7d
6	86.3 ± 6.3cd	76.3 ± 6.1c	99.0 ± 1.3a	99.0 ± 1.7a	99.0 ± 1.5c	72.5 ± 1.7c	86.3 ± 6.1e
7	86.3 ± 4.5cd	76.3 ± 4.5c	99.0 ± 1.5a	99.0 ± 1.3a	98.0 ± 1.4c	77.5 ± 5.2cd	93.8 ± 1.8f
8	88.8 ± 3.1cd	78.8 ± 3.7cd	99.0 ± 1.4a	99.0 ± 1.4a	99.0 ± 1.6c	77.5 ± 6.1cd	93.8 ± 1.1f
9	95.0 ± 2.7d	85.0 ± 2.7de	99.0 ± 1.3a	99.0 ± 1.6a	99.0 ± 1.3c	86.3 ± 5.1d	93.8 ± 1.5f
10	95.4 ± 2.2d	90.0 ± 2.4e	99.1 ± 1.5a	99.1 ± 1.3a	99.3 ± 1.1c	80.7 ± 5.4c	80.5 ±1.7de

Note. The differences between averages are significant at $P \leq 0.05$ in the absence of identical letters; the comparison was performed using the Tukey test.

Table 5. Effectiveness (%) of weeds control by herbicides in sunflower crops on 24 days after application in 2021 ($x \pm SE$; n = 4)

No.	ECHCG	SETPF	RAPRA	CAPBP	CHEAL	POLCO	VIOAR
2	59.9 ± 10.1bc	55.3 ± 10.1bc	99.5 ± 1.1a	99.7 ± 1.5a	98.6 ± 1.3b	70.6 ± 8.3a	95.6 ± 1.7b
3	75.5 ± 5.4c	70.2 ± 5.1c	99.4 ± 1.5a	99.4 ± 1.4a	99.4 ± 1.1b	91.4 ± 2.1bc	95.3 ± 1.5b
4	28.8 ± 10.1a	25.8 ± 10.4a	97.1 ± 2.5a	97.5 ± 2.3a	93.7 ± 2.3a	63.3 ± 8.7a	88.4 ± 2.5a
5	49.4 ± 10.1b	45.3 ± 10.2b	99.3 ± 1.5a	98.8 ± 1.5a	99.0 ± 1.4b	79.8 ±10.2b	98.9 ± 1.4c
6	94.3 ± 5.7d	90.6 ± 5.2d	99.1 ± 1.4a	98.9 ± 1.1a	99.5 ± 1.4b	98.4 ± 1.1c	98.8 ± 1.6c
7	95.7 ± 2.5d	95.0 ± 2.3d	99.0 ± 1.1a	98.9 ± 1.5a	99.6 ± 1.3b	99.1 ± 1.5c	98.8 ± 1.5c
8	98.7 ± 1.4d	99.0 ± 1.8d	98.8 ± 1.4a	98.9 ± 1.6a	99.2 ± 1.5b	99.6 ± 1.3c	99.6 ± 1.9c
9	98.8 ± 1.4d	99.1 ± 1.5d	99.0 ± 1.6a	98.6 ± 1.4a	98.9 ± 1.5b	99.5 ± 1.7c	98.9 ± 1.5c
10	99.0 ± 1.4d	98.6 ± 1.4d	98.8 ± 1.4a	99.0 ± 1.6a	94.3 ± 2.4a	97.6 ± 2.1c	94.7 ± 1.6b

Note: Explanation under Table 4

The data of Tables 4 and 5 show that herbicides aclonifen and prometryn, when applied alone, as well as the herbicide Primekstra, were highly effective in controlling annual dicotyledonous weeds mustard, wild radish, lamb's quarters, shepherd's purse. Due to the joint use of herbicides aclonifen and prometryn significantly, in comparison with the action of herbicides applied separately, increased the effectiveness of the control of annual dicotyledonous weeds black bindweed, birdweed, and field pansy, as well as annual grass weeds barnyard grass and yellow foxtail. The maximum controlling efficiency of the mixture of dicotyledonous weed species was achieved with the application rate of aclonifen 1.8 kg ha^{-1} and prometryn 1.5 kg ha^{-1}, or aclonifen 2.4 kg ha^{-1} and prometryn 1.0 kg ha^{-1}. Maximum efficiency of controlling of annual grass weeds in 2021 was achieved at the same application rates, while in 2020, There was a tendency to increase the effect on grass weeds at the maximum rates of application of the components of the mixture.

In the conditions of the 2020 year, the mixture of herbicides aclonifen and prometryn was only slightly inferior, and in the conditions of the 2021 year, did not differ from the action of the herbicide Primekstra on grass weeds. In terms of the effectiveness of controlling of annual dicotyledonous weeds birdweed and field pansy, the mixture slightly, but significantly exceeds the action of the herbicide Primekstra.

It is very important that in contrast to the greenhouse experiment, there were no signs of antagonistic interactions on grass weeds, when the aclonifen and prometryn herbicides were jointly applied. At all ratios of application rates of components, the expected effect did not exceed the actual (Figure 1). There was a clear tendency to exceed the actual effect of mixtures over the expected on birdweed (Figure 2) and black bindweed (Figure 3). Nevertheless, due to the rather high variability in determining the values of the expected action, this excess is not reliable.

Biometric measurements and phenological observations did not reveal a negative effect of herbicides on sunflower plants in any of the treatments of the experiment. Thus, it can be argued that the mixture of herbicides aclonifen and prometryn in the range of application rates 1.8–2.4 kg ha^{-1} and 1.0–1.5 kg ha^{-1}, respectively, is selective for sunflower. Confirmation of the selectivity of this mixture for crop is a significant increase in the yield of sunflower seeds (Table 6).

The use of the mixture of herbicides aclonifen and prometryn ensured reliable preservation of sunflower seed yield, which did not differ significantly from the action of the complex herbicide Primekstra TZ Gold, which was used as a reference. Practically equivalent efficiency of preservation of crop yield was observed at application of one component in smaller, and another in bigger rate: aclonifen of 1.8 kg ha^{-1} and prometryn of 1.5 kg ha^{-1} or aclonifen of 2.4 kg ha^{-1} and prometryn of 1.0 kg ha^{-1}. The use of both components in higher rates did not lead to a significant increase in the effectiveness of crop protection and a significant increase in the value of sunflower seed yield.

In contrast to the results of the greenhouse experiment, where at a certain ratio of application rates, the addition of aclonifen led to an antagonistic reduction of prometryn effect on sowing oats, as a model of annual grass weeds, in the field experiment, the interaction of aclonifen with prometryn on annual grass weeds barnyard grass and yellow foxtail was additive. This difference is probably due to the greater resistance to the studied herbicides of oat plants compared to barnyard grass and yellow foxtail. It is known that at the complex use of some herbicides, the character of their interaction may depend on the plant tolerance and, accordingly, on the magnitude of their phytotoxic action: at low values of phytotoxic action antagonistic interaction is more probable, and at higher values of phytotoxic influence the character of interaction, can change to additive or synergistic (Morderer & Merezhynsky, 2009). Although, the aclonifen and prome-

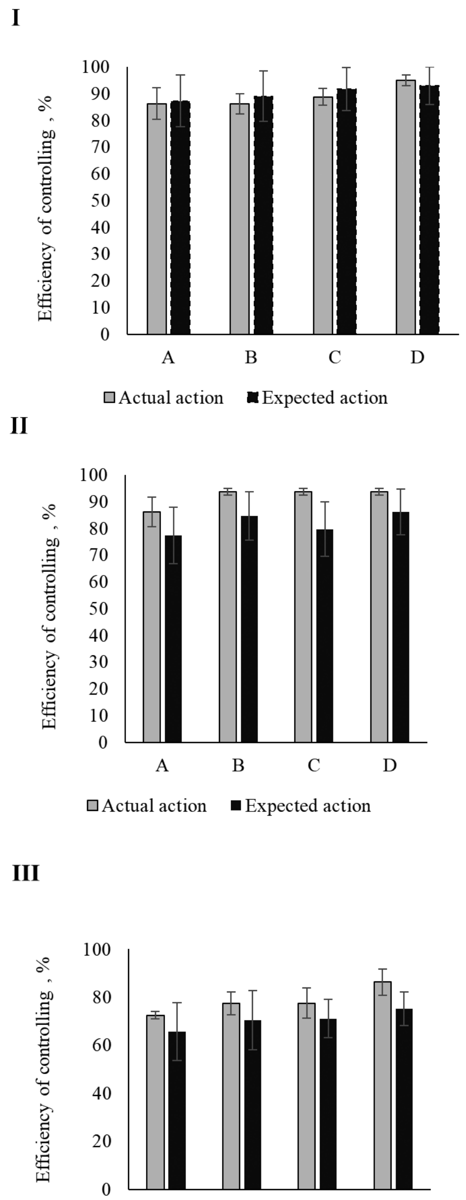

Fig. 1. Actual and expected efficiency (%) of controlling of annual grass weed barnyard grass (I), annual dicotyledonous weeds birdweed (II) and black bindweed (III) under the action of mixtures of herbicides aclonifen and prometryn

Note. A – Aclonifen (1.8 kg ha^{-1}) + Prometryn (1.0 kg ha^{-1}); B – Aclonifen (1.8 kg ha^{-1}) + Prometryn (1.5 kg ha^{-1}); C – Aclonifen (2.4 kg ha^{-1}) + Prometryn (1.0 kg ha^{-1}); D – Aclonifen (2.4 kg ha^{-1}) + Prometryn (1.5 kg ha^{-1}); x ± SE; n = 4; the comparison was performed using the Tukey test.

Table 6. Sunflower seed yield (t ha^{-1}) of Neoma hybrid when using herbicides and their complexes (x ± SE; n = 4)

No.	Treatments	2020	2021
1	Control	1.59 ± 0.04a	2.29 ± 0.11a
2	Aclonifen (1.8 kg ha^{-1})	2.60 ± 0.17b	3.47 ± 0.15b
3	Aclonifen (2.4 kg ha^{-1})	2.29 ± 0.10b	3.60 ± 0.16bc
4	Prometryn (1.0 kg ha^{-1})	2.45 ± 0.20b	3.32 ± 0.18b
5	Prometryn(1.5 kg ha^{-1})	2.31 ± 0.09b	3.65 ± 0.18bc
6	Aclonifen (1.8 kg ha^{-1}) + Prometryn (1.0 kg ha^{-1})	2.50 ± 0.18b	4.18 ± 0.21c
7	Aclonifen (1.8 kg ha^{-1}) + Prometryn (1.5 kg ha^{-1})	3.03 ± 0.16c	4.44 ± 0.20c
8	Aclonifen (2.4 kg ha^{-1}) + Prometryn (1.0 kg ha^{-1})	2.97 ± 0.14c	4.71 ± 0.23c
9	Aclonifen (2.4 kg ha^{-1}) + Prometryn (1.5 kg ha^{-1})	3.01 ± 0.21c	4.08 ± 0.15c
10	S-metolachlor (1.4 kg ha^{-1}) + terbuthylazine (0.84 kg ha^{-1})	3.06 ± 0.18c	4.09 ± 0.21c

Note: Explanation under Table 4.

tryn are mainly intended for the control of annual dicotyledonous weeds in sunflower crops, but the additive character of their interaction provided a fairly high efficiency of grass weeds control by the mixture of these herbicides, which is almost not inferior to the action of the complex herbicide Primekstra.

Expectations of the possibility of herbicides aclonifen and prometryn synergistic interaction, which were based on the data of synergism in the mixtures of HPPD inhibiting herbicides and herbicides ET inhibitors (Armel et al., 2008; Walsh et al., 2012; O'Brien et al., 2018; Osipitan et al., 2018; Willemse et al., 2021) did not find confirmation in our study. Increasing the phytotoxic effect of aclonifen in the greenhouse experiment on oilseed radish plants by the addition of prometryn, cannot be considered evidence of synergism. It is known that the Colby method can be used to assess the effects of interactions in herbicide mixtures, only if the inhibitory effect of the individual components of the mixture is close to 50%. In the case, when the effect of one of the components is weak and, accordingly, much lower than 50%, the increase in the phytotoxic effect of the mixture is more likely due to the nonlinear character of the concentration dependence of the action of herbicides (Blouin et al., 2004). In the field experiment, a significant proportion of dicotyledonous weeds were effectively controlled by the use of herbicides aclonifen and prometryn applied separately. The character of the interaction could only be assessed for grass and some species of dicotyledonous weeds, the control of which at the application of aclonifen and prometryn alone was close to 50%. In particular, for birdweed and black bindweed at all ratios of application rates of components, there was a tendency to exceed the actual effect of the mixture over the expected. Though, this excess was not very significant and due to the low accuracy in calculating the expected effect of the mixture, the difference between the actual and expected efficiency is not significant.

The difference in the character of the interaction in the mixture of aclonifen with prometryn from mixtures of HPPD inhibitors with ET inhibitors may be due to two factors. Firstly, synergism in mixtures of HPPD inhibitors with ET inhibitors was observed mainly after treatment of plant seedlings, whereas a mixture of herbicides aclonifen and prometryn was applied to the soil before the emergence of sunflower and weed seedlings. Secondly, the different character of the interaction may be due to differences in the mechanism of inhibition of carotenoid synthesis by aclonifen and HPPD inhibitors. HPPD inhibitors block the synthesis of plastoquinone, which is a cofactor of carotenoid biosynthesis (Lee et al., 1997). The mechanism of synergism in the mixtures of HPPD inhibitors with ET inhibitors can thus be due to two factors. First, increasing the efficiency of blocking ET, because plastoquinone is one of the elements of the electron transport chain, competing with ET inhibitors herbicides for the D1 protein binding site of the chloroplast PS 2 reaction center (Dan, 2000).

Second, HPPD inhibitors reduce the effectiveness of the plant protection system against damage by reactive oxygen species (ROS), not only by blocking the synthesis of carotenoids, but also due to inhibition of tocopherol production, which is a classic antioxidant that can block the development of lipid peroxidation reactions (Willis et al., 2007). Under the action of aclonifen, which inhibits the synthesis of carotenoids by inhibiting the desaturation reaction of phytoen due to blocking the activity of the enzyme SPS (Kahlau et al., 2020), possibility of direct influence on the effectiveness of ET inhibition is absent. In addition, the effect of aclonifen on the activity of the antioxidant protection system of plants is less than the action of HPPD inhibitors. Though, as our results show, the additive interaction provides on the one hand the selectivity of the mixture of herbicides aclonifen and prometryn for sunflower, and on the other hand, the quite high efficiency of controlling a wide range of weed species. At the same time, the advantage of this mixture is that it fully meets the requirements, necessary to prevent the emergence of herbicide-resistant weed biotypes (Norsworthy et al., 2012).

Conclusions

High efficiency of weed controlling in sunflower crops by the mixture of herbicides was achieved with the aclonifen and prometryn application rates, respectively, 1.8 and 1.5 kg ha^{-1}, or 2.4 and 1.0 kg ha^{-1}. The interaction of herbicides aclonifen and prometryn, when applied jointly to the soil, before the emergence of plant seedlings of sunflower is mainly additive. The mixture of herbicides is selective for sunflower in the range of application rates of aclonifen 1.8–2.4 kg ha^{-1} and of prometryn 1.0–1.5 kg ha^{-1}. Aclonifen and prometryn application rates that are effective for controlling grass weeds should obviously be higher than for controlling dicotyledonous weeds. Components of this mixture have different mode of action, but the range of weed species controlled by them significantly intersects.

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