

## Article

# Seed Meals from Allelopathic Crops as a Potential Bio-Based Herbicide on Herbicide-Susceptible and -Resistant Biotypes of Wild Oat (*Avena fatua* L.)

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**Abstract:** Wild oat is a nuisance monocot weed mainly in cereal canopies. The increasing problem of herbicide resistance and the implementation of integrated pest management includes the use of seed meals from species with allelopathic potential. This study aimed to assess the efficacy of seed meals as an environmentally friendly alternative for the control of herbicide-susceptible (S) and -resistant (R) wild oat biotypes. A pot experiment was carried out under greenhouse conditions at the Swojczyce Research and Training Station in Wrocław (Poland) to determine the impact of seed meals from *Fagopyrum esculentum*, *Lupinus luteus*, *Ornithopus sativus*, *Phacelia tanacetifolia*, *Raphanus sativus* var. *oleiformis* and *Sinapis alba* at two concentrations—1 and 3%. The lowest percentage of seedling emergence was recorded after the application of *S. alba* seed meal. Seed meals from *S. alba* reduced the emergence of both S and R biotypes by approximately 82 and 61 percentage points, respectively, and limited the initial growth of both biotypes. *F. esculentum*, *O. sativus* and *R. sativus* seed meals generally did not decrease the number of seedlings. Almost all the seed meals inhibited the growth of the aboveground parts of the susceptible biotype of wild oat to a greater extent than the herbicide treatment.

**Keywords:** non-chemical weed control; allelopathy; herbicide susceptible; herbicide-resistant; integrated weed management; weed control; monocots weed; efficacy



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## 1. Introduction

The problem of herbicide-resistant weeds is growing globally [1] and reducing the efficacy of herbicides [2–6]. Among all pests, it is weeds that reduce crop yields the most. At a global level, estimations indicate that weeds cause potential yield losses of around 34% in main crops, much greater than those caused by insects (18%) or diseases (16%) [7]. Increasingly frequently, herbicide-resistant biotypes of weeds are limiting crop production. The growth of their occurrence in arable crops is a major challenge for people involved in the agriculture and horticulture sectors, including farmers, crop production advisors, scientists, pesticide legislators and employees in the plant protection and seed industry [8]. They are one of the main threats to the biodiversity of agroecosystems [9]. The evolution of this problem and its complexity often leads to an increase in the costs of weed control too. Preserving the biodiversity of agroecosystems is one of the overarching goals of the Green Deal policy and sustainable development. Limiting and reducing the speed of development of resistance to herbicides is based on slowing selective pressure on resistance. This effect can be achieved by using different methods of weed control [10]. This is why it is important to look for alternative possibilities to control this group of pests. Out of concern for the environment, the highest standards of safety have to be maintained.

The use of biotechnical agents, including natural substances of plant origin, should play an important role in this regard [11]. These substances are biodegradable and do not place a burden on agroecosystems. Around 800 species of plants produce biologically active substances, but only a few of them are used in agricultural practice. They account for less than 1% of the market for plant protection agents, especially those for weed control. However, in recent years, there has been an increase in interest in allelopathy due to the effective use of allelochemicals in plant protection, such as bioherbicides, bioinsecticides, biofungicides and growth regulators [12,13]. This may be a result of the implementation of integrated plant protection and the need to implement sustainable development, including in agriculture. Allelochemicals may be found in different parts of plants, i.e., in the roots, leaves, stems and in the seeds [14]. They may alter a variety of physiological processes, such as cell division and differentiation, water and ion uptake, phytohormone metabolism, photosynthesis, respiration and enzyme function [15]. The production and release of allelocompounds by plants may affect other organisms, including plants, in the way they inhibit or stimulate their growth and development [16,17]. The way in which these compounds found in plants act is similar to that of synthetic herbicides. Due to the high variety of metabolites that they contain, it is possible to generate new and environmentally friendly natural herbicides [18,19]. Allelocompounds of plant origin are in most cases safe for humans and are not toxic to soil or water. Still, synthetic herbicides are very effective, though their overuse has a negative effect on human health and the environment [14].

In arable fields, one of the species of weed that is the most common and most difficult to control is wild oat (*Avena fatua* L.). It is an annual monocotyledonous weed that is particularly troublesome in cereal crops throughout Europe [20–24], North America [25–27] and Australia [28]. It is considered to be one of the most aggressive grass weeds found in spring cereal crops in Poland [29]. *A. fatua* can occur in other crops—winter cereals, root crops, legumes, vegetables and even ornamental crops. It may grow in grasslands or woodland communities [27]. In recent decades, there have been numerous reports of weed biotypes of wild oat that are resistant to herbicides. Although herbicides are still available for the control of *A. fatua*, resistance to several commonly used active ingredients is becoming more prevalent. To date, wild oat has evolved resistance to many herbicide modes of action, including acetyl-CoA carboxylase (ACCase) inhibitors (HRAC Group 1), acetolactate synthase (ALS) inhibitors (HRAC Group 2), enolpyruvyl shikimate phosphate synthase inhibitors (HRAC Group 9), very long-chain fatty acid synthesis inhibitors (HRAC Group 15), and cell elongation inhibitors (HRAC Group 0). On a global scale, numerous cases of multiple resistance of wild oats have also been observed. In the case of the ACCase and ALS inhibitor resistance, they are mainly connected to the use of herbicides with the same mode of action and a lack of crop rotation [1,22,23,30–33]. Despite the growing number of herbicide-resistant biotypes of wild oat, there are not many references in the literature to the possibility of using non-chemical methods, including those based on allelopathic compounds for the control of this species [34,35].

The aims of the research were (1) to assess the effect of seed meals from allelopathic crops on the emergence and initial development including biometrical, and physiological parameters of herbicide-susceptible or -resistant biotypes of wild oat; and (2) to compare the efficacy of wild oat control by seed meals with herbicide spraying.

The research hypothesis assumed that the addition of seed meals to the soil will limit the emergence and initial development of herbicide-susceptible and -resistant biotypes of wild oat.

## 2. Materials and Methods

### 2.1. Plant Materials

In a pot experiment, wild oat with varying susceptibility to herbicides was tested as the acceptor species (Table 1). The herbicide-susceptible (S) and -resistant (R) biotypes of wild oat were collected from spring wheat fields in July 2020. The R biotype was characterized by a low resistance index ( $2 \leq R \leq 4$ ) to propoxycarbazone-sodium.

**Table 1.** Characteristics of herbicide-susceptible (S) and -resistant (R) biotypes of wild oat (*A. fatua*) used in a pot experiment. ED50 values express the effective dose of propoxycarbazone-sodium (HRAC Group 2) causing a 50% reduction in plant biomass (ED50).

Biotype	ED50 (g ha <sup>-1</sup> )	Site (Coordinate)
S	14.75	Wrocław (51.1360 N 17.1150 E)
R	57.96	Środa Śląska (51.1767 N 16.6687 E)

## 2.2. Seed Meals and Their Preparation

The seed material of selected crop species (Table 2) was milled the day before the pot studies were started. All the selected commercial seeds were ground to meals in a Fritsch Pulverisette 11 laboratory mill (Idar-Oberstein, Germany). The seed meals were sealed in zip lock bags and stored in the fridge at a temperature of 5 °C. The species of crops for the preparation of seed meals were chosen based on our previous studies [17] and the literature [36–38].

**Table 2.** Crop species and cultivars used to prepare the seed meals [17].

Name		Cultivar	Abbreviation
English	Latin		
Common buckwheat	<i>Fagopyrum esculentum</i> Moench.	Panda	FE
White mustard	<i>Sinapis alba</i> L.	Bardena	SA
Lacy phacelia	<i>Phacelia tanacetifolia</i> Benth.	Anabela	PT
Yellow lupin	<i>Lupinus luteus</i> L.	Mister	LL
Fodder radish	<i>Raphanus sativus</i> L. var. <i>oleiformis</i> Pers.	Adagio	RS
Common birdsfoot	<i>Ornithopus sativus</i> Brot.	Bydgoska <sup>1</sup>	OS

<sup>1</sup> Variety not included in the national register.

## 2.3. Herbicide Characteristics

The active ingredient of the herbicide used in a pot experiment was propoxycarbazone-sodium (70%). According to the HRAC classification, it is classified as belonging to Herbicide MoA (Mode of Action) Group 2. The propoxycarbazone-sodium presents a systemic type of action. Users are recommended to apply it to leaves. A selective herbicide, which was used in experiments, is applied in the form of water-soluble granules (SG).

## 2.4. Soil Characteristics

The soil used in the pot experiment was formed from light loamy sand underlaid with poor loamy sand. It was classified as an IVb quality class of soil (in Poland equivalent to a good rye complex). The soil was characterized by the following parameters: pH<sub>KCl</sub> 5.84; P 85.7; K 28.1; Mg 134.0 (mg kg<sup>-1</sup> of soil) and content of organic matter of 1.13%. The soil was collected after harvesting the forecrop of organic forage pea cv. 'Roch'.

## 2.5. Set Up and Management of Pot Experiments

Two series of pot experiments were carried out in a greenhouse in 2020 and 2021. Series I began in November and series II started in March at the Wrocław University of Environmental and Life Sciences Research and Training Station in Swójczyce (southwest Poland). During the experiment, the lighting (12 h day/12 h night) and thermal (15 °C at day and 10 °C at night) conditions were regulated in both series. The plants were irrigated

as needed. The first factor studied was the type of seed meal; the second—the concentration of seed meal. Both acceptors (S and R biotypes of wild oat) were analyzed individually.

Before conducting a pot experiment, the soil was sieved over 1 cm mesh screens to rid the soil of post-harvest residue and stones. The experiment was carried out as a totally randomized design with 3 pots as replications. Production pots of 0.5 L in volume were filled up with a mixture of 500 g of sieved soil and one of the tested seed meals (from each species individually) in an amount of 1 or 3% (*w/w*). The control (C) and herbicide control (HC) pots did not incorporate any addition of seed meals. Ten grains each of either of the biotypes of wild oat (S or R) were sown into soil-filled pots. Seventeen days after sowing, when the weeds had 1 leaf (BBCH 11), the number of plants per pot was equalized to 5, if the number of seedlings permitted. The HC treatment was sprayed at the 2-leaves-unfolded stage (BBCH 12) of wild oat in the spray chamber (APORO sp. z o.o., Poznań, Poland). The dose of propoxycarbazone-sodium was 56 g ha<sup>-1</sup> 200 L H<sub>2</sub>O. Experiments were harvested when the plants in control treatment (without weed management) of wild oat were at the 4-leaves-unfolded stage (BBCH 14).

### 2.6. Measurement Range

Wild oat emergence was counted 14 days after sowing. During the harvest, the plants were pulled out and measured. The fresh weight of above- and belowground parts was determined (roots were washed and dried on a paper towel) using a WTC 2000 scale (RADWAG, Kraków, Poland). On this basis, the efficacy of biomass reduction in the tested treatments (seed meals at two concentrations and herbicide, separately) in relation to the control treatment was calculated. A minus value of the index indicates an increase in the mass of wild oat after incorporating seed meals into the soil or spraying herbicide on the leaves. The length of the aboveground parts of the plants (from the shoot base to the edge of the longest leaf) was measured. The relative chlorophyll content in leaves (in SPAD units) was measured on the youngest fully developed leaves on all plants in pot using a CCM-200 chlorophyll content meter (Opti-Sciences, Hudson, NH, USA). The efficiency of photosystem II was assessed by means of chlorophyll *a* fluorescence with a Handy Plant Efficiency Analyzer (PEA) chlorophyll fluorimeter from Hansatech Instruments Ltd. (King's Lynn, UK). The measurements were conducted after a 30 min adaptation of leaves on clips to darkness with an excitation light intensity of 3 mmol m<sup>-2</sup> s<sup>-1</sup> (peak wavelength 650 nm). The following measurements were taken in the experiment: Fv/Fm represents the maximum yield of photosystem II (PS II) and P.I.—overall performance index PS II. The measurements of leaf PSII efficiency were performed on the leaves in 2 replications (1 leaf per replication). The next day, the area of the aboveground parts of wild oat was measured using a CI-202 LASER LEAF AREA METER from CID Bio-Science (Camas, WA, USA).

### 2.7. Statistical Analysis

Statistical analysis was conducted, for both series combined, using the two-way variance analysis (type and concentration of seed meal) Statistica 13.3 software (TIBCO Software Inc., Tulsa, OK, USA). To check the normality of the distribution, the Shapiro–Wilk test was performed. The homogeneity of variance was checked using the Levene test. In order to determine and verify the relationships, Tukey's post hoc test was performed with a significance level of  $p \leq 0.05$ .

The results were also analyzed using multidimensional methods. Principal Component Analysis (PCA) was used to present a multidimensional assessment of the similarity of the tested combinations in a smaller number of dimensions with the least possible loss of information. For this purpose, the Kaiser rule was applied. Principal Component Analysis was carried out for the combinations of biotypes of wild oat and type, and concentration of the seed meal. The analysis was conducted based on the variables: above- and belowground biomass per one plant, aboveground plant area, SPAD index, and indicators of chlorophyll *a* fluorescence: Fv/Fm, PI. PCA made it possible to visualize the variability of biotypes of wild oat with regard to all the observed traits in graphical form.

### 3. Results and Discussion

The type and concentration of seed meals with allelopathic potential added to the soil caused differences in the number of wild oat seedlings of the S and the R biotypes (Table 3).

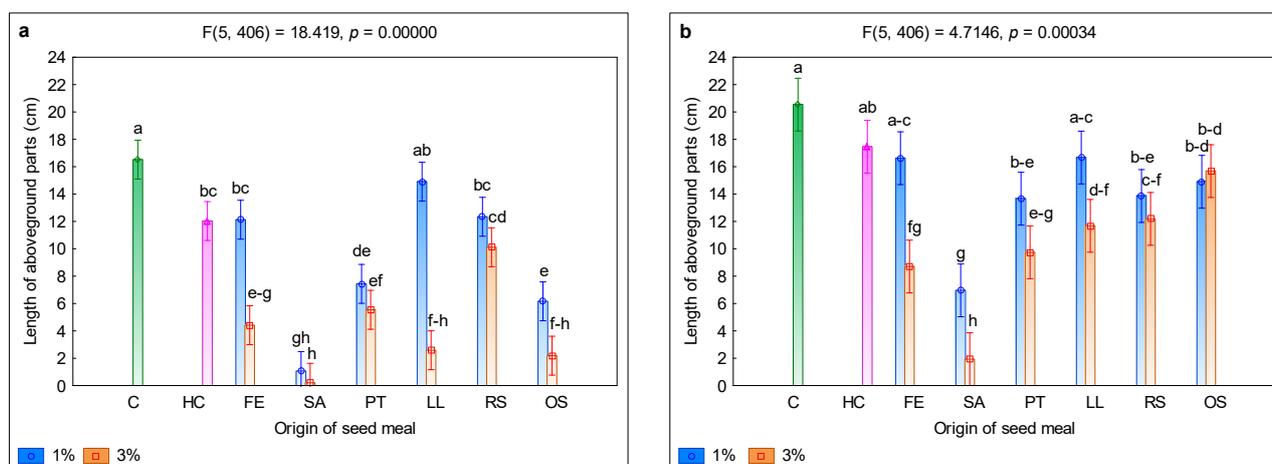
**Table 3.** The mean and the standard deviation emergence of herbicide-susceptible (S) and -resistant (R) biotypes of wild oat depending on the origin of seed meals and their concentration (14 days after sowing).

Origin of Seed Meals	Biotype	
	S	R
C	87.0 ± 4.7	83.3 ± 4.1
FE1	94.4 ± 2.6	81.5 ± 3.7
FE3	31.5 ± 4.2	51.9 ± 3.6
SA1	7.4 ± 1.5	35.2 ± 1.7
SA3	1.9 ± 0.6	9.3 ± 1.3
PT1	37.0 ± 2.5	50.0 ± 2.3
PT3	33.3 ± 4.0	48.1 ± 3.9
LL1	44.4 ± 2.9	66.7 ± 4.7
LL3	9.3 ± 2.5	55.6 ± 3.8
RS1	79.6 ± 6.7	77.8 ± 5.9
RS3	70.4 ± 3.2	79.6 ± 6.2
OS1	22.2 ± 3.6	74.1 ± 5.3
OS3	11.1 ± 0.6	66.7 ± 4.6

The symbols mean C—control and seed meals from FE—*Fagopyrum esculentum*, SA—*Sinapis alba*, PT—*Phacelia tanacetifolia*, LL—*Lupinus luteus*, RS—*Raphanus sativus*, OS—*Ornithopus sativus*; 1—1% concentration of seed meals, 3—3% concentration of seed meals.

The emergence of the S biotype was inhibited most weakly in the soil with the addition of RS1 and RS3 (*R. sativus*, 1 and 3%) seed meals relative to the control (C). The addition of FE1 (*F. esculentum*, 1%) seed meal led to an increase in the number of seedlings of the S biotype. The highest emergence of another monocotyledonous weed species—rye brome (*Bromus secalinus* L.)—after addition to soil seed meals from fodder radish (*R. sativus*) and buckwheat (*F. esculentum*) was also observed by [17]. The lowest percentage of seedling emergence was recorded after application of the SA (*S. alba*) seed meal, independently of the acceptor (S and R biotype). The authors [17,38,39] noted an inhibitory effect of seed meal from white mustard in relation to other species of monocotyledonous weeds, inter alia: rye brome (*B. secalinus*), smooth crabgrass (*Digitaria ischaemum* (Schreb.)), annual bluegrass (*Poa annua* L.) and fall panicgrass (*Panicum dichotomiflorum* Michx.). According to [40], glucosinolates and phenolic compounds contained in white mustard seeds may be responsible for the inhibition of growth. An endogenous enzyme—myrosinase—hydrolyzes glucosinolates, leading to the formation of a range of products with phytotoxic and herbicidal potential. The main products of this hydrolysis are isothiocyanates [41–43]. Unfortunately, the addition to the soil of white mustard seed meal may also limit the growth of crop species, such as common wheat [17], maize [36] and vegetables [37,38]. Moreover, in our research, in the case of the R biotype, the weaker response of emerging seedlings to the seed meal addition was observed. The FE3, PT1 (*P. tanacetifolia*), PT3 and LL3 (*L. luteus*) seed meals were fairly effective in limiting emergence (along with the SA seed meal). After their application, the percentage of seedling emergence for the R biotype of wild oat amounted to 52% (FE3), 50% (PT1), 48% (PT3) and 56% (LL3). With an increase in the concentration of seed meals, an increase in the limitation of seedling emergence was observed (except with the RS seed meal), though the difference between concentrations was not as big as in the case of the S biotype.

The seed meal application limited the average length of aboveground parts of the S (Figure 1a) and the R biotypes of wild oat (Figure 1b).

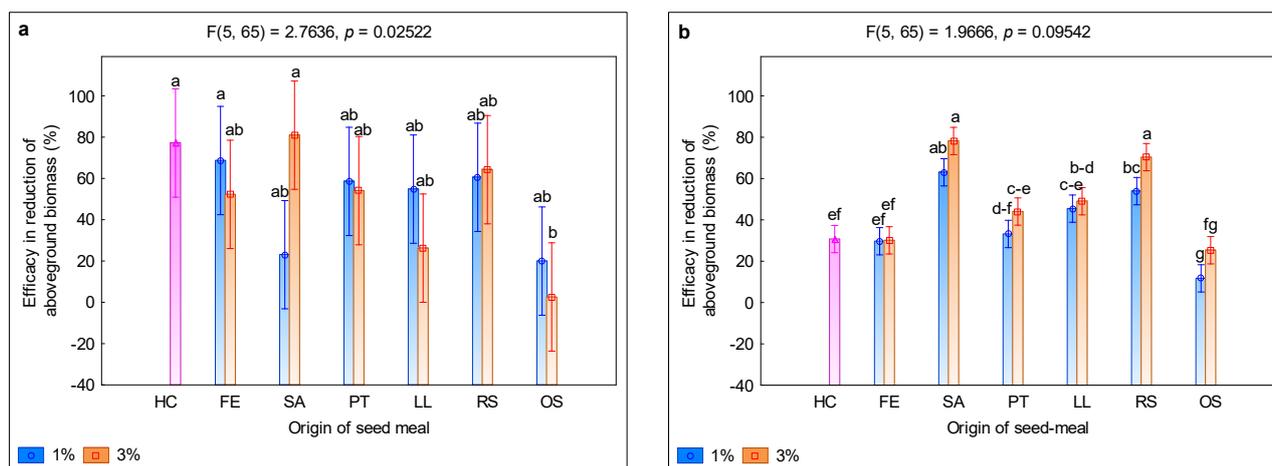


**Figure 1.** The mean length of aboveground parts of herbicide-susceptible (a) and -resistant (b) biotypes of wild oat depending on the origin of seed meals and their concentration. Means with various letters are significantly different, according to Tukey test ( $p \leq 0.05$ ). Vertical bars mean 0.95 confidence intervals. The symbols mean C—control, HC—herbicide control, and seed meals from FE—*Fagopyrum esculentum*, SA—*Sinapis alba*, PT—*Phacelia tanacetifolia*, LL—*Lupinus luteus*, RS—*Raphanus sativus*, OS—*Ornithopus sativus*. The column colors mean green—control, pink—herbicide control, blue—1% concentration of seed meals, orange—3% concentration of seed meals.

Compared with C, the addition of seed meals to the soil led to a significant reduction in the length of the aboveground parts of the S biotype of wild oat, excluding LL1. Furthermore, the application of nearly all the seed meals (except for LL1, RS1, RS3 and FE1) for the S biotype also caused a significant reduction in the length of the aboveground parts compared to HC. The SA seed meal proved to be most effective at limiting the length of the aboveground parts of both biotypes of wild oat. After the application of seed meal SA3, a shortening in the length of the aboveground parts of wild oat by 91–99% was observed, compared to C, for the R and S biotypes, respectively. Interestingly, in studies by other authors [41], after the addition of white mustard seed meal to the soil, a stimulatory effect on the growth of the acceptor—common wheat—was observed, which manifested itself in the formation by the plants of longer aboveground parts. Similarly to our results, the development of winter wheat and rye brome was strongly limited after the addition of white mustard seed meal to the soil [17]. Isothiocyanates released from the seeds during hydrolysis were probably responsible for this. It can be assumed that the seed meal from RS (another species of the *Brassicaceae* family) contains fewer glucosinolates and phenolic compounds, which is why its action is less effective than that of the SA meal [41,44]. In our own studies, after addition to the soil of seed meal SA1, an inhibitory effect on the length of aboveground parts compared to C (reduction in length by 66–93% for the R and S biotypes, respectively) was observed. An increase in the dose of seed meals from plants with allelopathic potential also resulted overall in a stronger inhibition of the growth of monocotyledonous weeds [17,36]. It is worth underlining that, in our studies, the SA seed meal also resulted in the greatest limitation of the emergence of both biotypes of wild oat (cf. Table 3). Significant growth inhibition of the aboveground parts of the S biotype of wild oat was also noted after addition to the soil of seed meals LL3 and OS3 (*O. sativus*). At the same time, there was found to be a reduction in the average length of the aboveground parts by 84–87%, respectively, compared to C. Some authors [45] point out that the genus *Ornithopus* may suppress weeds by releasing secondary metabolites with allelopathic potential into the soil. These compounds display a strong inhibitory activity in relation

to growing seedlings, especially those species of monocotyledonous weeds. According to [46], in-field and laboratory weed suppression was associated with the presence of several flavonoids and their glycosides, specifically quercetin, kaempferol, isoquercetin, and kaempferol-7-O-glucoside.

The efficacy of seed meal SA3 in the reduction in aboveground biomass of the S biotype of wild oat was four percentage points (p.p) higher than the efficacy of spraying with herbicide, and was slightly over 80% (Figure 2a).



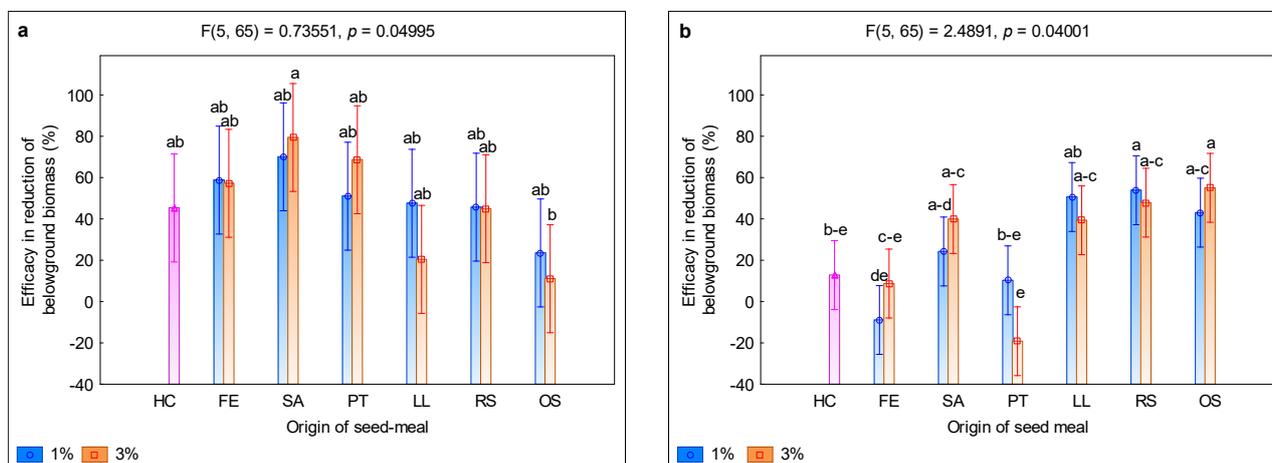
**Figure 2.** The mean efficacy in reduction in aboveground biomass of herbicide-susceptible (a) and -resistant (b) biotypes of wild oat depending on the origin of seed meals and their concentration. Means with various letters are significantly different, according to Tukey test ( $p \leq 0.05$ ). Vertical bars mean 0.95 confidence intervals. The symbols mean HC—herbicide control, and seed meals from FE—*Fagopyrum esculentum*, SA—*Sinapis alba*, PT—*Phacelia tanacetifolia*, LL—*Lupinus luteus*, RS—*Raphanus sativus*, OS—*Ornithopus sativus*. The column colors mean pink—herbicide control, blue—1% concentration of seed meals, orange—3% concentration of seed meals.

In turn, after the addition of the seed meal OS3 to the soil, the efficacy of the limitation of the biomass of the S biotype aboveground parts was found to be at its lowest. It only amounted to just under 3% and was significantly lower than with HC, the seed meals of FE1 and SA3 by 74, 66 and 78 p.p., respectively. Many authors [47–49] draw attention to root exudates of common buckwheat suppressing weed development. Moreover, [17] have found meals from common buckwheat seeds to be highly effective at limiting aboveground biomass in the S and R biotypes of rye brome. As in our own studies, the concentration of that seed meal did not make any difference to the efficacy of the limitation of the aboveground biomass of the species tested.

For the R biotype, after application of the SA seed meal (at both concentrations), a significantly higher efficacy in the reduction in aboveground biomass was observed compared to HC treatment (Figure 2b). The average efficacy in limiting aboveground biomass after using herbicide was then only 31%, while the addition of seed meals SA1 and SA3 to the soil resulted in an increase in efficiency by 32 and 47 p.p., respectively. As [41,44] point out, isothiocyanates released into the soil during the hydrolysis of glucosinolates from seeds of the *Brassicaceae* have a herbicidal effect. Dazomet—an active ingredient used as a means of plant protection—breaks down in the soil and releases methyl isothiocyanate, which limits the development and occurrence of pests, including weeds. In the case of the R biotype, nearly all the seed meals applied limited the growth in aboveground biomass to a level that was at least comparable to that with herbicides. In addition to the SA seed meal, significantly higher efficacy, compared to HC, was also noted for the other seed meal obtained from a plant belonging to the *Brassicaceae* family, i.e., RS. After application of the seed meal RS1, efficacy in the reduction in aboveground biomass of the R biotype of wild oat was 54%, while with RS3, it was 70%. Our results are consistent with [41] who also

underline a strong reduction (by 55%) in the dry weight of wild oat after addition to the soil of white mustard seed meal at a dose of 40 g kg<sup>-1</sup> of soil (concentration 4%).

Addition to the soil of seed meals did not result in any significant difference in the development of the belowground parts of the S biotype of wild oat, compared to the HC (Figure 3a).



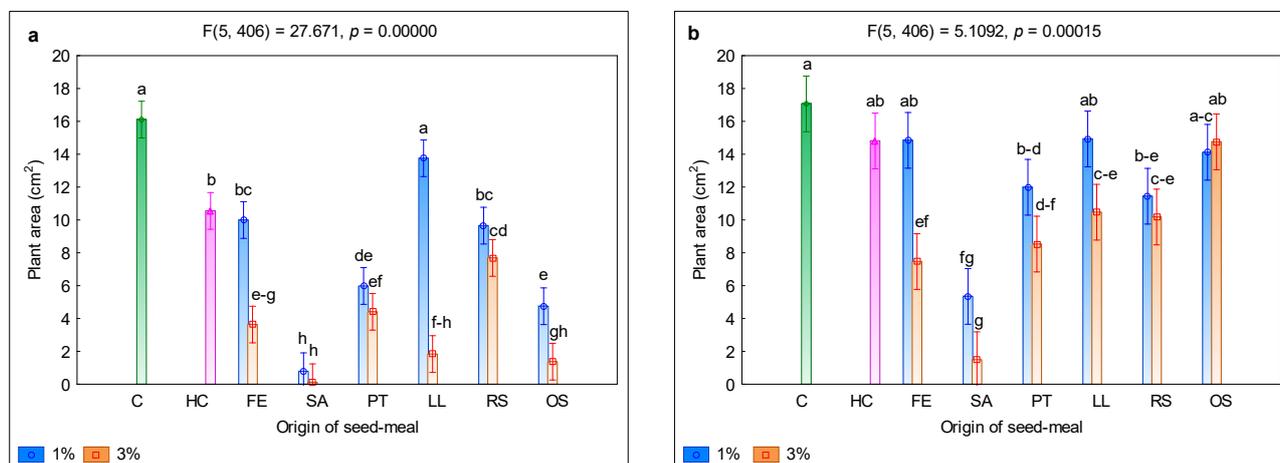
**Figure 3.** The mean efficacy in reduction in belowground biomass of herbicide-susceptible (a) and -resistant (b) biotypes of wild oat depending on the origin of seed meals and their concentration. Means with various letters are significantly different, according to Tukey test ( $p \leq 0.05$ ). Vertical bars mean 0.95 confidence intervals. The symbols mean HC—herbicide control, and seed meals from FE—*Fagopyrum esculentum*, SA—*Sinapis alba*, PT—*Phacelia tanacetifolia*, LL—*Lupinus luteus*, RS—*Raphanus sativus*, OS—*Ornithopus sativus*. The column colors mean pink—herbicide control, blue—1% concentration of seed meals, orange—3% concentration of seed meals.

However, it can be noticed, the most effective was the seed meal SA3. Its efficacy was as high as 80% and was nearly twice as high as that of treatment with herbicide. It should be underlined that the seed meal SA3 was also the most effective at reducing emergence (cf. Table 3) and growth in the aboveground biomass of the tested biotype *A. fatua* (cf. Figure 1a). The higher efficacy of seed meals applied to the soil compared to spraying with herbicide may result from the fact that the allelopathic compounds released from the seed meals had an effect on the plants for a longer period than the active ingredient of the herbicide. Studies such as [17] support this claim—the authors found that seed meals limited the development of root biomass of rye brome more strongly than herbicides. This may mean that the biologically active substances contained in white mustard seeds have a strong inhibitory action in relation to wild oat, even at low concentrations. Interestingly, similar observations concerning the lack of any significant difference in the effect of seed meals SA1 and SA3 were also made for the remaining biometric parameters, discussed above, of the S biotype of wild oat (cf. Figures 1a and 2a).

In the case of the R biotype, seed meals PT and FE were the least effective at reducing belowground biomass (Figure 3b). However, they achieved the same level of efficacy as after spraying with herbicide. Our finding is supported by [17], who in studies on the control of the R rye brome noted lower efficacy in the limitation of root development after addition to the soil of seed meal PT. The reduced efficacy of seed meal FE at limiting this parameter may result from its lower content of quercetin, which is responsible for the herbicidal action of buckwheat. Quercetin content is dependent on weather conditions in the period of development of buckwheat seeds [47]. In our own studies, the addition to the soil of all the remaining seed meals resulted in the limitation of the growth of aboveground biomass to a greater degree than spraying with herbicide. The SA seed meal, which had the highest efficacy in the case of the S biotype, was also highly effective at limiting the fresh weight of the belowground parts. Its efficacy was 40–55%, for concentrations of 1 and

3%, respectively, while the efficacy of the treatment with herbicide was only approximately 13%. Once again, no significant difference was found regarding the impact on belowground biomass between seed meals SA1 and SA3.

The surface area of the aboveground parts of the S biotype of wild oat was significantly lower than in the C, except for seed meal LL1 (Figure 4a).

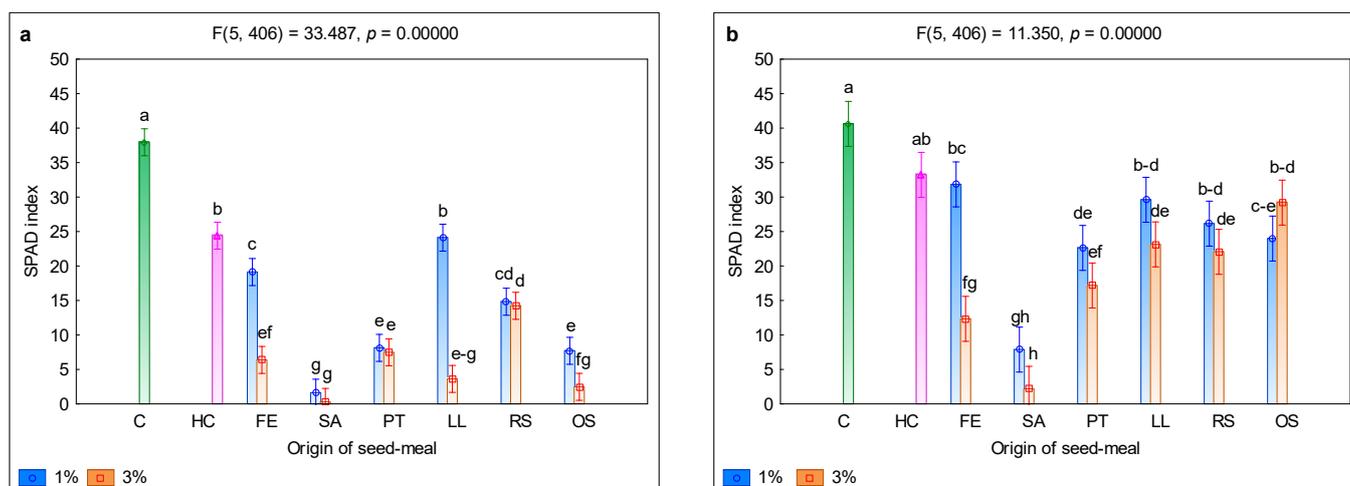


**Figure 4.** The mean aboveground surface area of herbicide-susceptible (a) and -resistant (b) biotypes of wild oat depending on the origin of seed meals and their concentration. Means with various letters are significantly different, according to Tukey test ( $p \leq 0.05$ ). Vertical bars mean 0.95 confidence intervals. The symbols mean C—control, HC—herbicide control, and seed meals from FE—*Fagopyrum esculentum*, SA—*Sinapis alba*, PT—*Phacelia tanacetifolia*, LL—*Lupinus luteus*, RS—*Raphanus sativus*, OS—*Ornithopus sativus*. The column colors mean green—control, pink—herbicide control, blue—1% concentration of seed meals, orange—3% concentration of seed meals.

The surface area of the aboveground parts was only 0.12–0.80 cm<sup>2</sup>, for seed meals SA3 and SA1, respectively; while in the C, it was over 16 cm<sup>2</sup>. In addition, the majority of the seed meals limited the surface area of the S biotype significantly more than spraying with herbicide, except for the aforementioned LL1 seed meal, as well as FE1 and RS1.

In the case of the R biotype, the significantly smallest surface area of the aboveground parts, compared to all other treatments, was once again observed for the SA seed meal (Figure 4b). In the SA3 treatment, the average surface area was just under 2 cm<sup>2</sup> and was 8 times smaller than in the C (17 cm<sup>2</sup>) and 7 times smaller than in HC (15 cm<sup>2</sup>). The rest of the seed meals at 3% concentration were more effective at limiting the surface area of the aboveground parts than C and HC, except for seed meal OS3. For all of the tested parameters, the response of the R biotype was less pronounced than that of the S biotype (cf. Figures 1–3). However, the application of the SA seed meal significantly limited the growth of plants in each case. Studies by other authors [17,50,51] point to the reduction in leaf surface area after exposure of the plant to the effects of stress. Some species of plant react to stress with changes in leaf area without any loss of biomass. This morphometric parameter may, therefore, in certain species of plants, be a sensitive indicator of a stress situation, including the presence in the environment of allelopathic substances. In our study, plants of limited weight also produced a smaller surface area of aboveground parts.

The addition to the soil of seed meals resulted in a significant reduction in the SPAD index in the S biotype of wild oat, in comparison to the C (Figure 5a).



**Figure 5.** The mean SPAD index of herbicide-susceptible (a) and -resistant (b) biotypes of wild oat depending on the origin of seed meals and their concentration. Means with various letters are significantly different, according to Tukey test ( $p \leq 0.05$ ). Vertical bars mean 0.95 confidence intervals. The symbols mean C—control, HC—herbicide control, and seed meals from FE—*Fagopyrum esculentum*, SA—*Sinapis alba*, PT—*Phacelia tanacetifolia*, LL—*Lupinus luteus*, RS—*Raphanus sativus*, OS—*Ornithopus sativus*. The column colors mean green—control, pink—herbicide control, blue—1% concentration of seed meals, orange—3% concentration of seed meals.

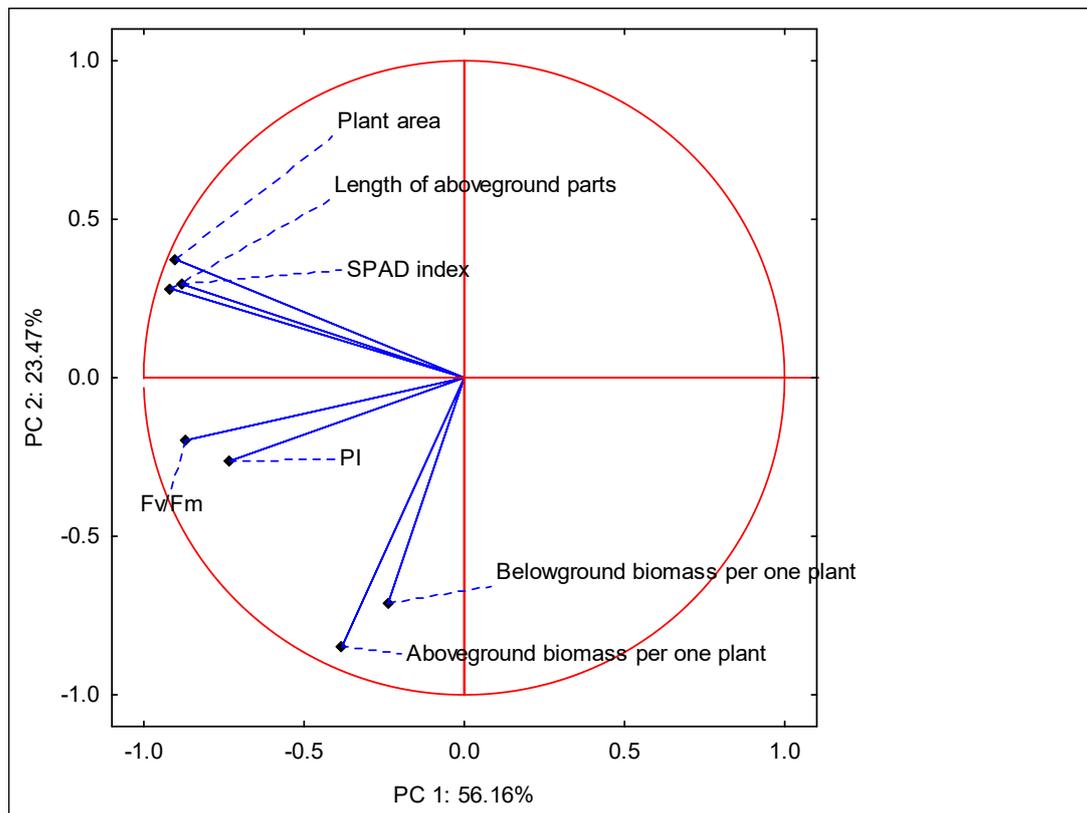
Application of the herbicide resulted in a reduction in the value of the SPAD index, compared to the C, at the same level as with the seed meal LL1 (approximately 36%). An increase in the concentration in the soil of seed meals SA, PT and RS did not result in any significant decrease in the value of the SPAD index. This once again confirms the strong inhibitory action of the selected seed meals even at low concentrations. Our finding is supported by [36], who studied the impact of the addition of seed meals to the soil on the SPAD index for *E. crus-galli*. The value of the SPAD index of the tested species with an increase in the concentration of seed meals from *S. alba* and *R. raphanistrum* did not decrease. The leaf chlorophyll content is an important indicator of the photosynthetic capacity [52,53]. In combination with the leaf area index, it has been found to be a critical indicator for vegetation productivity [54] and incidence stress in vegetation [55].

The R biotype of wild oat responded with a significant decrease in the SPAD index after the addition to the soil of each of the tested seed meals, in comparison to the C (Figure 5b). There was also found to be a significant difference between the HC and the seed meals at a concentration of 3% (except for seed meal OS3). Here, once again, it was the SA seed meal that proved to be most effective at inhibiting the growth of wild oat. A decrease in the SPAD index by 33–39 units was noted, for seed meals SA1 and SA3, respectively.

Following the Kaiser rule, the first two principal component axes were retained, which together accounted for 79.63% of the total variance. The following variables were the most strongly correlated with the axis of the first factor: length and area of the aboveground parts, SPAD index and  $F_v/F_m$ , while the most correlated with the second axis was the biomass of the aboveground parts (Table 4; Figure 6). However, [56] point out that the performance index (PI) is a good indicator of stress in plants, and [50] emphasize the role of leaf area in this respect. However, it should be emphasized that the  $F_v/F_m$  parameter, which is strongly correlated with the axis of the first factor, may not indicate plant stress occurring in the roots [57].

**Table 4.** Set of eigenvalues together with percentage share of total variance of principal components and factor loading of variables for PCA taking into account the growth and physiological traits of herbicide-susceptible and -resistant biotypes of wild oat.

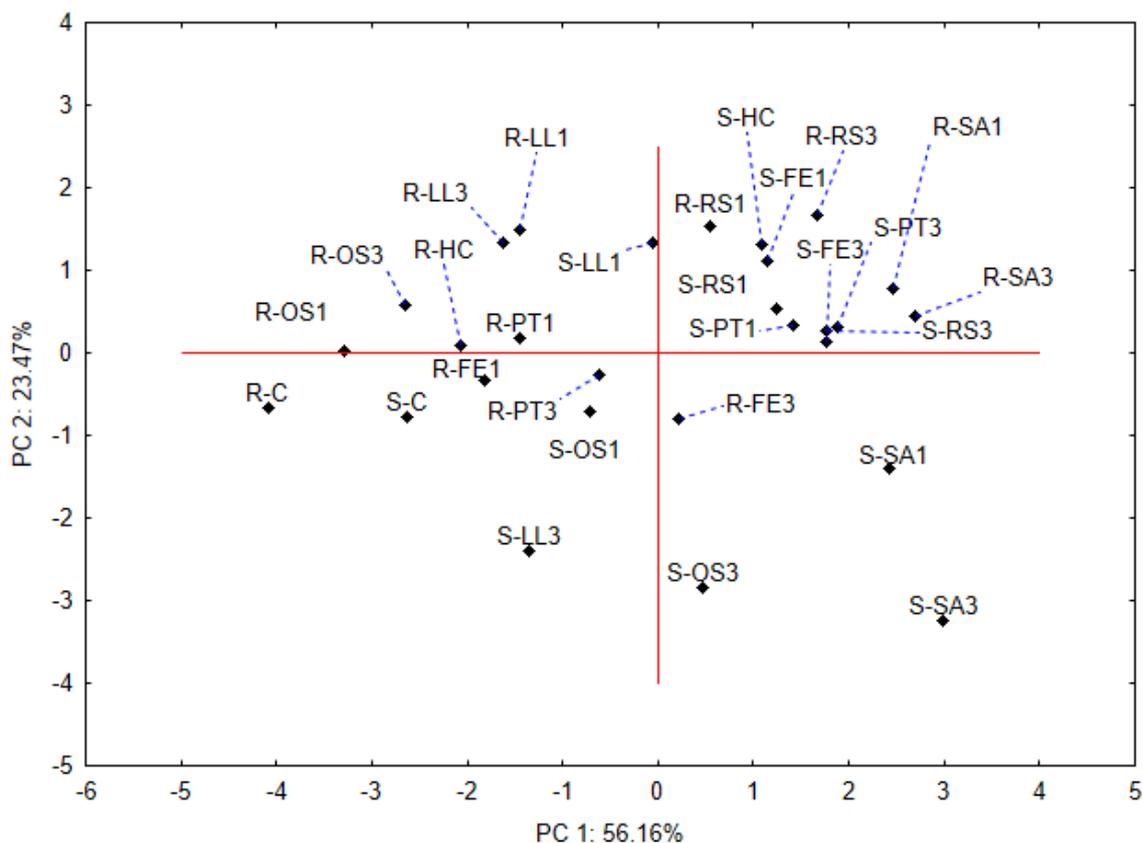
PARAMETER	PC 1	PC 2
Eigenvalues	3.93	1.64
% of total variance	56.16	23.47
Variables	Factor loadings of variables	
Aboveground biomass per one plant	−0.382	−0.849
Belowground biomass per one plant	−0.239	−0.713
Plant area	−0.901	0.374
SPAD index	−0.884	0.296
Fv/Fm	−0.869	−0.197
PI	−0.732	−0.262
Length of aboveground parts	−0.919	0.282



**Figure 6.** PCA analysis chart for the growth and physiology of herbicide-susceptible and -resistant biotypes of wild oat.

The distribution of the tested weed control variants in relation to the axis (PC 1 and PC 2) does not show clear sets of individual combinations (Figure 7). However, the majority of them (11 out of all 28 combinations) are concentrated in the area of the minimum values of both axes in the top right of the chart. This applies to both the S and R biotypes of wild oat. A good weed-limiting effect can be obtained in the S-HC combination (biotype susceptible to propoxycarbazone-sodium after application of herbicide). All variants with the RS seed meal of both biotypes of wild oat are to be found there, as well as the R biotype with seed meal from the other species of the *Brassicaceae* family—SA. Based on a PCA

analysis, [36] also found white mustard seed meal to be highly effective at controlling *Amaranthus retroflexus*, and to be of reduced efficacy in the case of *E. crus-galli*. Along the axis representing the value of the first principal component (PC 1), which is most decisive with regard to total variance, together with an increase in the values of the parameters: area, length and SPAD, the proportion of the R biotypes increases in relation to the S, which in turn differ in terms of their position on the axis representing the value of the second principal component (PC 2).



**Figure 7.** Projection of results of PCA analysis for the growth and physiological traits of biotypes of wild oat with seed meals of different species and at different concentrations in the soil. The individual letters show the order of the tested variants in the space of the first two principal components. The symbols mean S—herbicide-susceptible biotype, R—herbicide-resistant biotype, C—control, HC—herbicide control, and seed meals from FE—*Fagopyrum esculentum*, SA—*Sinapis alba*, PT—*Phacelia tanacetifolia*, LL—*Lupinus luteus*, RS—*Raphanus sativus*, OS—*Ornithopus sativus*, 1—1% concentration of seed meals, 3—3% concentration of seed meals.

#### 4. Conclusions

The study found that selected seed meals with allelopathic potential from crop species can constitute an alternative to herbicide management strategies for the control of herbicide-susceptible and -resistant (to propoxycarbazone-sodium) biotypes of wild oat. The addition of SA (*S. alba*) seed meal to the soil at both concentrations (1 or 3%) strongly inhibits the emergence and initial development of both biotypes of weed, including with regard to their biometric and physiological parameters. The seed meals: FE1 (*F. esculentum*) and RS1 (*R. sativus*) for the herbicide-susceptible biotype and FE1, RS1, RS3 and OS1 (*O. sativus*) for the -resistant biotype, do not clearly limit weed emergence. Future research should concentrate on a more comprehensive examination of seed meals in weed management by looking at other herbicides, other levels of herbicide susceptibility and even resistance, and considering other species of weeds and crops.

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