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Research Article

How long can an aryloxyphenoxypropionate herbicide be kept in the tank without losing efficacy?

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Abstract: Sometimes, the spray solution must be stored for some time. This study aimed to answer the following questions: how long can an aryloxyphenoxypropionate herbicide be kept in the tank without losing efficacy? Is it dependent on pH? The experiment was designed as a completely randomized design with three factors including the labeled dose of herbicides (clodinafop, cyhalofop, diclofop, fenoxaprop, fluazifop, haloxyfop, propaquizafop, and quizalofop), the pH of spray solution (5, 7, and 8), and the storage time of spray solution (0, 12, 24, 36, 48, 60, and 72 h). The efficacy of herbicides on winter wild oat *Avena sterilis* subsp. *ludoviciana* Durieu. was not affected by changing the pH of the spray solution when the spray solution was applied immediately after preparation. When the spray solutions were stored, the herbicides' highest and lowest efficacy were generally observed at pH 5 and 9, respectively. At pH 9, except for fluazifop and haloxyfop, the effectiveness of other herbicides was reduced after 12-h storage of spray solution. The efficacy of all herbicides was reduced with 24-h spray solution storage. At pH 7, the efficacy of cyhalofop and fluazifop remained stable with 72-h storage of their spray solution. In contrast, at pH 5, the efficacy of clodinafop, cyhalofop, diclofop, fluazifop, haloxyfop, and quizalofop remained stable with 72-h storage of their spray solution. Therefore, if it is necessary to keep the spray solution of aryloxyphenoxypropionate herbicides for a short time, it is recommended to use a lower pH to avoid reducing their efficacy.

Keywords: alkaline water, chemical degradation, hydrolysis, winter wild oat

Introduction

The spraying operation may be delayed after adding the herbicide to the tank due to an unforeseen event. In such a situation, the spray solution must be kept in the tank for several hours or even days. For example, high wind speed exo-drifting the spray solution to the non-target area, heavy rainfall shortly after spraying endo-drifting the spray solution from the leaf surface into the soil, and application equipment failure (Schortgen and Patton, 2020) can also stop the spraying operation. Sometimes, farmers deliberately prepare and store the spray solution in the tank in the evening to apply it in the early morning (Stewart *et al*., 2009). Previous studies have shown that an herbicide stored in the tank can be hydrolyzed or react with the compounds inside water, reducing the efficacy of herbicides in controlling weeds. Eight-day storage of glyphosate, lactofen, 2,4-D, atrazine,

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imazethapyr, clethodim, and dicamba spray solutions decreased control of *Urochloa subquadripara*, while it did not affect the efficacy of paraquat (Eure *et al*., 2013). Three-day storage of 2,4-D spray solution did not affect its efficacy against *Taraxacum officinale* (Schortgen and Patton, 2020). The efficacy of the premix formulation of diuron + carfentrazone was not affected when the spray solution was stored for nine days (Eure *et al*., 2011).

In contrast, one-day storage of the spray solution prepared separately from each herbicide showed a reduced efficacy. Stewart *et al*. (2009) did not observe a reduction in weed control efficacy with seven-day storage of diflufenzopyr, glyphosate, glufosinate, mesotrione, atrazine, nicosulfuron, and rimsulfuron solutions. Nalewaja *et al*. (1994) showed that the longer sethoxydim is stored in hard water, the lower its efficacy against *Setaria glauca*. They attributed the decreased efficacy of sethoxydim to its binding to cations in hard water (not hydrolysis). The binding between isoxaflutole and hypochlorite in drinking water when the spray solution was stored for one day has been observed to reduce herbicide efficacy (Lin *et al*., 2003).

Nevertheless, the seven-day storage of isoxaflutole + atrazine spray solution undid the negative effect of hypochlorite in drinking water on the efficacy of isoxaflutole (Stewart *et al*., 2009). Boerboom (2004) reported that one-day storage of dicamba spray solution reduces its efficacy. In addition, they showed that dicamba can bind to the wall of the sprayer tank. Silveira *et al*. (2020) reported that the nicosulfuron spray solution becomes more acidic with time, and three-day storage reduces its efficacy in controlling *Urochloa brizantha* by 30%.

The first question is how long an aryloxyphenoxypropionate (AOPP) herbicide can be kept in the tank without losing efficacy? Ten herbicides belonging to the chemical family AOPPs have been registered to date, including clodinafop, cyhalofop, diclofop, fenoxaprop, fenthiaprop, fluazifop, haloxyfop, metamifop, propaquizafop, and quizalofop (WSSA, 2014). The AOPPs can inhibit the enzyme acetyl CoA carboxylase (ACCase) in the fatty acid biosynthesis pathway, disrupting cell division and growth in grassy species and leading to their death. In the aquatic environment, the ester bond of AOPPs can abiotically hydrolyze over time to their corresponding AOPP acid. Then, AOPP acid is re-hydrolyzed to form some metabolites, leading to the lack of weed control (Lamberth and Dinges, 2016). For this reason, the standing spray solution of AOPP should be thrown away.

The metabolites derived from the hydrolysis of fluazifop (Badawi *et al*., 2015) and quizalofop (Buerge *et al*., 2020) can leach faster to the surface and subsurface waters than the parent herbicide. The half-life of the metabolites derived from fenoxaprop is longer than the parent herbicide (Jing *et al*., 2016). On the other hand, the metabolites derived from AOPPs have been reported to be more toxic than the parent herbicide.) The metabolites derived from fenoxaprop have higher toxicity to *Daphnia magna*, an aquatic organism, than the fenoxaprop itself (Jing *et al*. 2007; Lin *et al*. 2008). Similarly,) The metabolites derived from diclofop have higher toxicity to *Chlorella pyrenoidosa* - a freshwater microalga than the diclofop itself (Cai *et al*. 2007). Abd-Alrahman *et al*. (2014) studied the accumulation of diclofop and its metabolites in the muscle tissue of *Oreochromis niloticus*, a freshwater fish. They reported that only the metabolites derived from diclofop can accumulate in the muscle tissue, not diclofop. Therefore, throwing the spray solution of AOPPs away when the application is delayed is environmentally undesirable. In addition, it is also economically undesirable. The second question is, what is a solution to decelerate the hydrolysis process of AOPPs in the tank to avoid throwing it away when the application is delayed? Previous studies have shown that the rate of hydrolysis is affected by light and pH. Although the rate of hydrolysis of AOPPs (clodinafop (Roy and Singh, 2005), fluazifop (Balah *et al*., 2017), and haloxyfop (Harrison and Wax, 1986)) has been reported to be accelerated by light, their response to pH has been reported to be different; so that the lowest rate of hydrolysis of metamifop (Saha *et al*., 2018) has been reported at alkaline pH, that of propaquizafop (Hazra *et al*., 2015) at neutral pH, and for quizalofop and clodinafop (Ahemad and Khan, 2009) at acidic pH.

The present study was carried out with the following objectives: 1) the effect of the storage time of AOPPs spray solutions on their efficacy against winter wild oat (*Avena sterilis* subsp. *Ludoviciana* Durieu.) and 2) the effect of pH on the above relationship.

Materials and Methods

This study was carried out in the early fall of 2021 in the Research Greenhouse of Bu-Ali Sina University in Hamedan, Iran. Panicles of winter wild oats were collected from the university campus in the previous spring and were kept in the room. The outer layers of the seeds (lemma and palea) were manually removed. After surface-sterilizing in 5% sodium hypochlorite solution for 1 min, they were placed from the grooved side on a layer of filter paper inside plastic Petri dishes with a diameter of 10 cm (around 50 seeds in each Petri dish). Then, 10 ml of 2 g potassium nitrate L^{-1} was added to each Petri dish. Firstly, they were placed in the refrigerator at 4 °C for 48 h; then at room temperature for 48 h. All steps of keeping the seeds were done in the dark. Five seedlings (germinated seeds) with a coleoptile of about 1 cm were transplanted into each pot, they were placed on the soil surface of the pots that had already been watered, and about 1 cm of soil was poured on them. With the appearance of the coleoptile and before its tearing, about 1 cm of soil was again poured on them. The size of the pots, brown plastic with a square section, was 13 × 13 × 13 cm. According to the area of the pots, the planting density was about 200 plants m^{-2}. The soil used for the seedbed had a ratio of 40:1 of soil: animal manure, respectively. The pots were watered uniformly every six days. During the experiment, air temperature and relative humidity inside the greenhouse were measured at 15-26°C and 32-59%, respectively.

The experiment was designed as a completely randomized three-factor (8 by 3 by 7) design. The first factor was the labeled dose of herbicides, including 64 g ha^{-1} clodinafop-propargyl (Topek 8% EC), 100 g ha^{-1} cyhalofop-butyl (Clincher 10% EC), 900 g ha^{-1} diclofop-methyl (Illoxan 34.7% EC), 75 g ha^{-1} fenoxaprop-p-ethyl (Puma Super 7.5% EW), 225 g ha^{-1} fluazifop-p-butyl (Fusilade 12.5% EC), 108 g ha^{-1} haloxyfop-r-methyl (Gallant Super 10.5% EC), 150 g ha^{-1} propaquizafop (Agil 10% EC), and 200 g ha^{-1} quizalofop-p-ethyl (Targa 5% EC). The second factor was pH of the spray solution, including 5, 7, and 8. Since Lin *et al*. (2003) reported that urban drinking water harms the efficacy of isoxaflutole due to its content of hypochlorite, in the current study, distilled water was used, which had a pH of 7 and its pH was adjusted to 5 and 8 using citric acid and sodium hydroxide, respectively. The third factor was the storage time of the spray solution, including 0, 12, 24, 36, 48, 60, and 72 h. The spray solution corresponding to 0 h was prepared and applied on the same day of spraying. Other corresponding spray solutions were prepared with a volume of 1 L at 12, 24, 36, 48, 60, and 72 h before the day of spraying) and stored in transparent polyethylene terephthalate plastic bottles. The bottles were kept under natural environmental conditions outside the greenhouse. During the storage period of the bottles, air temperature and relative humidity inside the greenhouse were measured at 11-21 °C and 35-47%, respectively. The treatments were applied at the 4-leaf stage of winter wild oat under open-air conditions (air temperature: 14-21 °C, relative humidity: 26-51%, and wind speed: 0-0.4 m s^{-1}) using a battery-powered backpack sprayer equipped with an 11002 Even Flat Fan nozzle and calibrated for 230 L ha^{-1} at a pressure of 3 bar. After spraying, the pots were again placed inside the greenhouse. A treatment of control without herbicide application was also considered. Three weeks after treatment, the shoots of the plants were removed from the soil surface, their fresh weight was weighed, and then their dry weight was weighed after two days of drying in the oven at 70 °C. The obtained data were divided by the number of plants in each pot, and the fresh-to-dry weight ratio was statistically analyzed. The fresh-to-dry weight ratio of winter wild oat shows the degree of burning the plant against herbicides. When the value of this ratio is equal to one, it indicates that the entire surface of the shoots is dried. In other words, the lower the value of this ratio, the greater the herbicide activity (Rytwo and Tropp, 2001).

Data normality was tested, and their normal distribution was stabilized (1 > Shapiro-Wilk > 0.9). Data were subjected to analysis of variance (ANOVA) using PROC GLM in SAS software version 9.4. The means were separated by Duncan's multiple range test at the 0.05 probability level.

Results

The fresh-to-dry weight ratio of winter wild oats non-treated (without herbicide) was measured to be 6.1. Since the *P*-value less than 0.01 in ANOVA showed statistical significance for three-way interaction (herbicide × pH × storage time) on the fresh-to-dry weight ratio of winter wild oat, the means for each herbicide level were separated using the SLICE option to illustrate the results (Fig. 1).

When the spray solution of herbicides was applied immediately after preparation (storage time = 0 h), the results showed the efficacy of the herbicides was not affected by changing the pH of the spray solution. The above treatments measured a fresh-to-dry weight ratio of winter wild oat between 1.2 and 1.8. In general, increasing the storage time of the spray solution of the herbicides increased the fresh-to-dry weight ratio of winter wild oat, indicating a decreased herbicide efficacy. When the spray solution was stored, the highest and the lowest values of the fresh-to-dry weight ratio of winter wild oat were generally observed at pH 9 and 5, respectively.

At pH 9, except for fluazifop and haloxyfop, a significant decrease in the efficacy of other tested herbicides was observed with 12-h storage of their spray solution. However, the efficacy of all herbicides was significantly reduced when their spray solution was stored at pH 9 for 24 h.

At pH 7, the efficacy of 2 of the 8 herbicides tested (cyhalofop and fluazifop) remained stable (without reduction) with 72-h storage of their spray solution. However, keeping the spray solution of clodinafop and fenoxaprop for 12 h, propaquizafop for 24 h, haloxyfop for 36 h, quizalofop for 48 h, and diclofop for 60 h at pH 7 reduced their efficacy against winter wild oat.

At pH 5, the efficacy of 6 of the 8 herbicides tested (clodinafop, cyhalofop, diclofop, fluazifop, haloxyfop, and quizalofop) remained stable (without reduction) with 72-h storage of their spray solution. However, keeping the spray solution of propaquizafop for 24 h and fenoxaprop for 48 h at pH 5 reduced their efficacy against winter wild oat.

Discussion

It has been reported that pH dose not affect the efficacy of some AOPPs. For example, quizalofop, fenoxaprop (McMullan,1996), haloxyfop, and fluazifop (Aliverdi *et al*., 2023). The reason is related to the formulation of AOPPs, which are formulated as pre-herbicides. An ester part (ethyl, methyl, butyl, etc.) is attached to the herbicide molecule. Hence, they remain electrically uncharged in the spray solution and do not react to pH changes (McMullan,1996). However, there is a report that the pH of the spray solution does not affect the efficacy of cyclohexanedione herbicides, which inhibit the ACCase in the fatty acid biosynthesis pathway (Bridges, 1989).

The hydrolysis of AOPPs has been proven in several studies (Harrison and Wax, 1986; Roy and Singh, 2005; Cai *et al*., 2007; Lin *et al*., 2008; Ahemad and Khan, 2009; Hazra *et al*., 2015; Balah *et al*., 2017; Saha *et al*., 2018). According to PubChem - an open chemistry database, the half-lives of AOPPs in water depend on pH (Table 1).

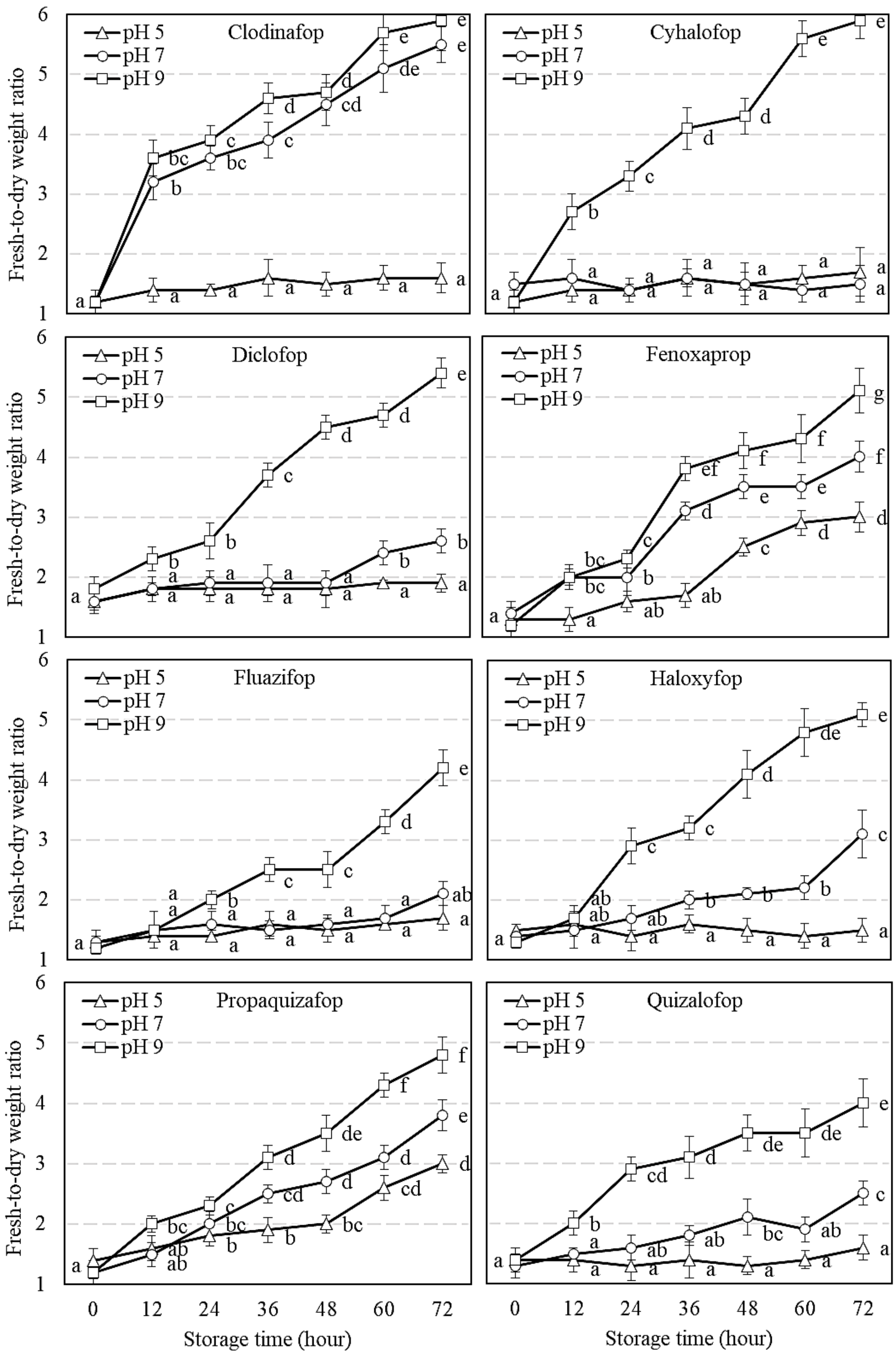

Figure 1 Fresh-to-dry weight ratio of winter wild oats treated with eight aryloxyphenoxypropionate herbicides when their spray solutions were prepared in three pHs and stored for 0-72 h. The fresh-to-dry weight ratio of winter wild oats non-treated was 6.1. Vertical bars are the standard error of the mean. The means followed by the same letter are not significantly different according to Duncan's multiple range test at the 5% probability level.

Table 1 shows higher stability of AOPPs has been generally observed in water with acidic pH followed by neutral and alkaline pH. For example, clodinafop in water with pH 5, 7, and 9 has a half-life of 184 days, 2.7 days, and 2.2 hours, respectively. When the AOPPs spray solution is stored, the ester bond of AOPPs can hydrolyze over time to their corresponding AOPP acid. Then, it is re-hydrolyzed to form some metabolites, leading to the lack of weed control (Lamberth and Dinges, 2016; Aliverdi *et al*., 2023). For this reason, the storage period of the AOPPs spray solution without losing efficacy can be increased by decreasing the pH (Fig. 1).

Table 1 Half-lives of AOPPs in different water pH.

AOPPs	pH 5	pH 7	pH 9
Clodinafop	184 days	2.7 days	2.2 hours
Cyhalofop	Stable	97 days	2.1 days
Diclofop	363 days	31.7 days	12.5 hours
Fenoxaprop	19.2 days	23.2 days	14.4 hours
Fluazifop	32 days	78 days	29 hours
Haloxyfop	Stable	43 days	15.1 hours
Propaquizafop	10.5 days	32 days	12.9 hours
Quizalofop	277 days	18.2 days	7.2 hours

AOPPs: aryloxyphenoxypropionate, Data is obtained from PubChem.

The bottles used to keep the spray solutions have been placed under natural environmental conditions outside the greenhouse. Therefore, they were exposed to light during the day. Since some AOPPs, such as fluazifop (Li *et al*., 2019), are sensitive to photodegradation and hydrolyze faster in the presence of light, keeping the spray solution of AOPPs in the dark can probably lead to an increase in their storage period without losing efficacy. As a possible future study, this must be backed up by data.

Li *et al*. (2021) reported that the photodegradation of fluazifop depends on the presence and type of the cations in water so that the cations of Mg^{2+} and Sn^{2+} can accelerate the photodegradation of fluazifop, while Co^{2+} and Li^{+} can stop it. Similarly, the presence of Co^{2+} and Ti^{4+} in spray solution has been reported to accelerate the photodegradation of atrazine (Chan and Chu, 2009) and fluazifop (Li *et al*., 2019), respectively. In our study, distilled water was used to prepare the spray solution of AOPPs, while in field conditions, the water used to prepare the spray solution of AOPPs usually contains some cations accelerating or stopping the photodegradation of AOPPs. Therefore, this study should be repeated for regions with different water conditions.

Conclusion

Based on the results of this study, the preparation and storage of the spray solution of AOPPs reduced their efficacy, which depended on the duration of storage and pH. Most of Iran's agricultural waters have a high pH, reducing the storage period of the spray solution of AOPPs without losing efficacy. In such a situation, the farmer may throw away the spray solution of AOPPs due to the loss of efficacy. Although AOPPs-derived metabolites do not have herbicidal effects, they have properties that cause some environmental problems. For example, they are more leachable, toxic, and bio-accumulable and have a longer half-life than the parent AOPPs. Therefore, if it is necessary to keep the spray solution of AOPPs for a short time (1 to 3 days, depending on the AOPP), it is recommended to use a pH reducer and keep it in the dark to avoid reducing its efficacy.

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چه مدت می‌توان علف‌کش آریلوکسی‌فنوکسی‌پروپیونات‌ی را بدون اُفت کارایی در مخزن نگه‌داشت؟

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چکیده: گاهی اوقات، محلول پاشش باید برای مدتی نگه‌داری شود. هدف از این مطالعه پاسخ به سؤالات زیر بود: علف‌کش آریلوکسی فنوکسی پروپیونات را تا چه مدت می‌توان بدون از دست دادن کارایی‌شان در مخزن نگه‌داری کرد؟ آیا این موضوع به pH بستگی دارد؟ این آزمایش به‌صورت طرح کاملاً تصادفی با سه فاکتور شامل دُز برچسب علف‌کش‌ها (کلودینافوپ، سی‌هالوفوپ، دیکلوفوپ، فنوکساپروپ، فلوآزیفوپ، هالوکسی‌فوپ، پروپاکویزآفوپ و کویزآلوفوپ)، pH محلول پاشش (۵، ۷ و ۸) و زمان نگه‌داری محلول پاشش (۰، ۱۲، ۲۴، ۳۶، ۴۸، ۶۰ و ۷۲ ساعت) به اجرا درآمد. وقتی محلول پاشش بلافاصله پس از آماده‌سازی اعمال شد، کارایی علف‌کش‌ها روی یولاف وحشی زمستانه *Avena sterilis* subsp. *ludoviciana* Durieu. تحت تأثیر تغییر pH محلول پاشش قرار نگرفت. وقتی محلول‌های پاشش ذخیره شدند، بیش‌ترین و کم‌ترین کارایی علف‌کش‌ها به‌ترتیب در pHهای ۵ و ۹ مشاهده شد. در ۹ = pH، به‌جز فلوآزیفوپ و هالوکسی‌فوپ، کارایی سایر علف‌کش‌ها با نگه‌داری ۱۲ ساعته محلول پاشش کاهش یافت. درحالی‌که کارایی تمامی علف‌کش‌ها با نگه‌داری ۲۴ ساعته محلول پاشش کاهش یافت. در ۷ = pH، کارایی سی‌هالوفوپ و فلوآزیفوپ با نگه‌داری ۷۲ ساعته محلول پاشش ثابت ماند، درحالی‌که در pH=۵، کارایی کلودینافوپ، سی‌هالوفوپ، دیکلوفوپ، فلوآزیفوپ، هالوکسی‌فوپ و کویزآلوفوپ با نگه‌داری ۷۲ ساعته محلول پاشش ثابت ماند. بنابراین، اگر اجباراً محلول پاشش علف‌کش‌های آریلوکسی‌فنوکسی‌پروپیونات‌ی باید برای مدت زمان کوتاهی نگه‌داری شود، توصیه می‌شود برای جلوگیری از کاهش کارایی آن‌ها از یک کاهنده pH استفاده شود.

واژگان کلیدی: آبکافت، آب قلیایی، تجزیه شیمیایی، یولاف وحشی زمستانه