Effect of reduced rate herbicides and citowett mixtures on weed control, oil composition and seed viability in canola

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ABSTRACT: Farm experiments were conducted to determine the effect of reduced rates of Butisan Star and Clopyralid with a given amount of Citowett on rapeseed associated weeds, oil composition, and seed viability in 2009. The trial was laid out in randomized complete block design (RCBD) with 11 treatments and four replications. The effects of treatments were assessed at the 2 leaf stage development of canola. Average grain yield in plots treated with mixture of 2/3 full rate Clopyralid and Citowett were similar to those plots which were treated with full rate of standard treatments. Addition of Citowett in a tank mix with Clopyralid substantially improved control of weeds which resulted in more grain yield. Canola oil quality was, generally, unaffected by production practices investigated. The contents of saturated fatty acids in the oil was ≈ 8.0% indicating that the quality of oil from canola produced in Urmia is comparable to that from other locations. Application of Clopyralid at 2 kg a.i. /ha and Citowett at 400 ml /ha effectively controlled weeds in canola, and significantly increased seed yields similar to those of standards; the herbicides mixture with Citowett had little, if any, effect on seed-oil content or oil quality. Canola oil quality was, generally unaffected by production practices investigated. The combination of two herbicides at reduced rate had no phytotoxic effect on the crop. Canola germination rate was not diminished by toxic impact of herbicides mixture with Citowett. The plumule length was not reduced following exposure to Clopyralid and Citowett mixture in comparison to untreated check.
[Ahad Motallebi, Mehdi Tajbakhsh and Ali Asghr Pourmirza. **Effect of reduced rate herbicides and citowett mixtures on weed control, oil composition and seed viability in canola.** *Life Sci J* 2012;9(3):1942-1950]. (ISSN: 1097-8135). http://www.lifesciencesite.com. 281
KEY WORDS: Reduced rate, herbicide, weed control, germination, fatty acid.

Introduction

Canola (*Brassica napus* L.) is a specific edible type of rapeseed, developed in the 1970s (Kandel & Knodel, 2011), which contains about 40 percent oil. Canola varieties must have an erucic acid content of less than 2 percent and less than 30 micromoles of glucosinolates per gram of seed. This makes it acceptable as an edible oil and animal protein feed. Canola oil is considered one of the highest quality edible oils available. Canola oil has achieved worldwide commodity status and is extensively used in Japan, Canada and Europe. Canola seeds contain 38-44% (w/w) oil (based on 8% moisture) with very low levels of saturated fatty acids (~ 6%), high levels of oleic acid (~ 60%) and intermediate levels of linoleic and linolenic acids (~20 and 10%, respectively) (Alternative Crop Overview, 2002).

There is general agreement that in the last two decades consumers have demanded healthier edible oils, naturally low in saturated fat such as olive and canola oils. The quality of oils is associated with their fatty acids (FA) composition, particularly with respect to the percentages of oleic (omega-9), linoleic (omega-6) and linolenic (omega-3) acids (Jalilian et al., 2012).

Heavy weed infestations left uncontrolled can reduce canola yields by 50% or more and can reduce seed quality leading to dockage (Gunsolus and Porter, 2004). Judicious weed management in canola should involve assessing weed species and weed densities present in each field and designing an integrated control program. Application of appropriate herbicide in weed control could be the most powerful tool to secure a high yielding, high quality crop of canola. Weed control strategies in canola, whether mechanical, chemical or a combination of both control methods should primarily be directed toward reducing weed competition during the first four to eight weeks after seeding. Otherwise, canola seedlings, which tend to grow slowly, can be overcome by certain weed species. Control of key broadleaf weeds was the most important constraint to production of canola throughout Iran.

Herbicide mixtures are considered powerful tools for cost effective control in intensive agriculture. A number of factors, however, may significantly modify the expected behavior of herbicide mixtures in practice. The selection of the most appropriate combinations should be made taking into account the properties of the herbicides to be combined and the species to be controlled.

Applying two or more herbicides simultaneously, either using prepackages mixtures or by mixing different herbicide products before the application, is a very common approach in intensive agriculture (Zhang et al., 1995). This is because the application of a single herbicide, even though may provide good control of certain weeds, is often inadequate for satisfactory and cost effective weed control. Furthermore, many herbicides have a narrow spectrum of weed control; whereas, other herbicides do not show the same efficacy against all weeds of their spectrum of control when applied at the recommended rates. Given that weed flora normally consists of many species with varying levels of herbicide sensitivity, more herbicide applications should be often performed or additional measures for weed control should be additionally adopted. This, however, increases the cost of weed control and consequently the cost of crop production. Good weed control is necessary for a good canola stand. Sod fields or fields with significant weed pressure should have weeds controlled in the fall prior to canola planting.

O'Donovan et al. (2004) stated that herbicide rate is an influential and crucial factor in weed control systems. This view is seconded by Shimi et al. (2007) who evaluated new herbicides in canola and reported that Butisan Top at 2-3 L/ha and Teridox at 2-3 L/ha are appropriate herbicides for weed control in canola fields. Despite what appears to be the sound basis for the use of mixtures as herbicide resistance prevention strategies, they have seldom been advocated or implemented. This is in great part due to the belief that in order to be effective, components of the mixtures must be used at the normal label rate (Diggle et al. 2003). A mixture tailored to prevent resistance would therefore, not only address the compatibility environmental requirement, but ensure that target weed species are adequately controlled.

To date, there are 313 resistant biotypes of weeds in over 270 000 fields worldwide (Heap, 2007). There are many tactics that are recommended to prevent or delay the evolution of herbicide resistance and diminish the threat of multiple-resistance in weeds. Among these tactics, the application of herbicide mixtures poses the outmost importance. Using mixtures does not necessarily mean that the overall chemical load is increased. It is conceivable that, because of additive or synergistic action among different molecules, the amount of each component could be reduced compared to when they are used alone at the full rate.

Ijaz et al. (2008) using several pre and post emergence herbicides in canola stated that none of the herbicides had a phytotoxic effect on the crop; the consequence of application of all herbicide is increased of canola yield. However, the post emergence herbicide is more effective when there is a preponderance of grassy weeds in infestation the crop. This view is seconded by Marwat et al. (2005) who reported that Treflan 4EC 1-2 liters ha^{-1} in rapeseed was the best treatment, reducing significantly the weed density and dry weight.

Considerable research has examined the potential use of lower-than-labeled herbicide doses. Kirkland et al. (2000) reported that good crop yields and the highest net returns could be attained up to a 50% herbicide dose in the crops with high competitive ability. Increasing the crop seed rate can be a dependable means of improving the efficacy of herbicides applied at reduced doses. In this line, the weed control was markedly improved with increased crop seed rates in a canola when a reduced rate of herbicides was utilized (O'Donovan et al., 2004).

Mixtures of herbicides with surfactants have been recommended for weed control enhancement and prevention of resistance phenomenon. Progress continues to be made on maintaining high efficacy levels at lower herbicide rates through the use of improved adjuvant that increase solubility in the spray tank and aid in herbicide uptake and translocation (Green and Cahill, 2003; Ramsey et al., 2005). Citowett as a non-ionic surfactant could be able to increase foliar uptake of active ingredients for example, by enhancing retention of spray droplets on cuticles and penetration and absorption into leaf tissue. An appropriate adjuvant assures maximum performance and crop safety. Curran et al. (1999) stated that the type of adjuvant added to the spray tank can enhance or reduce the performance of the pesticide. Progress continues to be made on maintaining high efficacy levels at lower herbicide doses through the use of improved adjuvant that increase solubility in the spray tank and aid in herbicide uptake and translocation (Green and Cahill 2003; Ramsey et al. 2005).

Seed germination tests provides the maximum germination potential of a seed lot and this can be used to compare the quality of different seed lots and also estimate the field planting value. For preservation of oil seeds quality, it is essential that the seed is tolerant to the herbicide spray solution. Seed viability could be a good indicator of oilseed quality after exposure to weed control agent. Therefore, in this study three measurements related to seed quality were investigated (ISTA, 1976).

The effects of reduced - rate of herbicides in combination with surfactants on crop has been reported previously. However, little information is available on the effects of reduced rate herbicides alone or in combination with Citowett on weed control, oil composition and canola seed viability. With retrospect, to fill this void and to have insight in this arena, the current research was undertaken.

Materials and Methods

Study Site

The field experiments were conducted at the research fields of Urmia University, Urmia (37.34° N 44.58° E and altitude 1365 m) a town in Iran in 2009. Soil of experimental site was sandy loam with pH of 7.1 and 1.5% organic matter. The trials were laid out in using randomized complete blocks design (RCBD) with 11 treatments including an untreated check and four replications with a plot size of 4 m × 3 m separated from adjacent plots by a 1 meter wide buffer. In order to have a uniform weed stand in each plot, a mixture of 100 g of *Xanthium pensylvanicum* Wall. and 50 g from each the rest common broadleaf rapeseed weed species and 7.2 g of canola seeds were sown by hand evenly as much as possible. Post emergence herbicides -Butisan Star, and Clopyralid- alone or in combination with Citowett were used. Addition of Citowett in a tank mix with herbicides was at a concentration of 0.1 % (v/v). Herbicides treatments were applied by Xinfeng Bao, 45 psi and 3 bar personnel, back pack sprayers and hand held booms equipped with fan nozzles. The treatments were sprayed at 400 L/ha of spray mixture. All trials were replicated four times.

In all plots of canola each experimental unit was harvested by hand. All canola samples collected in each bag were cleaned, weighed and tested for percent moisture content. Yields were calculated allowing for seed moisture content of 9%. Weed biomass from all experimental units was taken at harvesting time using a total quadrant area of 1 m^2 . For all experimental units, weeds were clipped at the ground level, identified, counted and placed in paper bags. Weeds were then dried for 2 weeks under ambient conditions and dry weights were measured. The combination mixture was compared to four standard herbicide treatments each composed of 2 herbicides at the full label rate to determine efficacy of the combination treatment. These treatments were applied in post-emergence of canola when the crop was at the two -leaf stage.

Oil Extraction and Composition

After the canola plant reached physiological maturity status, seed yield was secured by harvesting of one m^2 in the center of each plot. A sample of 20 g of clean seeds from each treatment was isolated to measure the oil concentrations. Soxhlet extraction technique was employed to determine the total oil concentration of the canola seed and the oil concentration was expressed as mg g^{-1} (Movahhedy-Dehnavy et al., 2009). The oil concentrations were reported as percent of the seed weight standardized to 9% moisture. Lipid was extracted from 20 g of ground seed three times at room temperature by homogenization with hexane/isopropanol (3:2, v/v) (St. John and Bell, 1989).

The formation of FAME was carried out according to the procedure described by Desvilettes et al. (1994**).** The sample was saponified with methanolic sodium hydroxide and the fatty acids were esterified with methanolic sulfuric acid. FAME were analyzed with a 6890 N GC–FID (Agilent Technologies, Wilmington, DE, USA) fitted with a J&W DB-Wax capillary column (30m, 0.25 mm i.d., 0.25 mm film thickness), a split–splitless injector with Agilent tapered liner (4 mm id) and flame ionization detector. The initial column temperature was maintained at 100°C for 1 min and then raised at 25°C/min to 190°C and held for 10 min. Nitrogen was used as carrier and make up gas, at flow rates of 1.0 and 45 mL/min, respectively. The injector and detector temperature were held at 250 and 260°C, respectively. ChemStation software was used for online data collection and processing. Individual FAME was identified by comparison with known standards (Sigma, Chemical Co. St. Louis).

Germination Tests

Germination tests were conducted according to the principles stated in International Seed Testing Association (ISTA, 1976) methods with minor modification. Seeds of oilseed were exposed to herbicide for 24 h in 1150 mL glass jar. Fifty herbicide treated seeds were soaked with 50 mL of distilled water for 24 h. Pre- treated seeds were spaced uniformly on sheet paper and placed in a germination cabinet for 8 d at 20°C. Non-herbicide exposed seeds treated identically and served as control standards for comparison. Each experiment was replicated four times on four different days. The number of germinated seeds was counted after four and eight days and the mean plumule length of fifty seedlings was determined at eight days.

Data Analysis

An arcsine square root transformation of the original data was performed to satisfy the assumptions that the error was random and normally distributed, and that all components were additive. Weed control and germination tests data were analyzed by analysis of variance (ANOVA) with SPSS software (SPSS Inc. 1993) followed by Tukey's Honestly Significant Difference (HSD) test to determine statistical differences between means at test ($P \leq 0.05$). For comparison to independent groups' means, T-test was used.

Results:

Treatments

Citowett as a surfactant was used in combination with treatments displayed in Table 1. Therefore, it was doted in a concentration of 250 ppm in tank mix spray solutions. Preliminary experiments revealed undetectable phytotoxic effects of Citowett on crop stands at recommended rate. As reference, herbicides solutions were applied alone and in combination with Citowett (Table 1).

Seed and Weed Weight

Statistical analysis of the data revealed that herbicides had significant effect on seed yield and weed weight (Table 2). The highest seed weight was obtained from standard treatments and mixture of Clopyralid and Citowett ranging 28.5 – 21.5 g. It was further observed that the lowest seed weight (3.5 g) was obtained from untreated check plots.

There was no significant effect of the treatments on crop injury or crop yields; while significant differences were observed among treatment seed yields and weed weights (Table 2). Together, crop yield was highly influenced by competition from weeds. In this context, an inverse relationship between seed yield and weed weight was detected. Therefore, in analysis of variance for the regression of seed yield on weed weight, mean seed yield was modeled as a function of weed's weight with linear equation: Seed yield = 31.28 – 0.087 weed weight. The equation has potential to be useful in prediction of the seed yield with $R^2 = 0.77$.

In the majority of cases, the mixture of 2/3 full rate of Clopyralid and Citowett at recommended rate (treatment 3) provided high level of seed yield and broadleaf weed control similar to the standard herbicides –recommended rate of Butisan Star and Clopyralid- (Table 3). A pronounced and significant difference was detected between resultant seed yield and weed control level of these treatments with the other treatments group. Addition of Citowett in a tank mix with both herbicides and associated reduced rate mixtures substantially improved of canola seed yield in comparison to treatment with lack of due Citowett addition (Table 3).

Dominant Weeds

The results indicated that among the weeds population, the *Echinchola crusgalli* L. and *Setaria faberil* Hernm. are significantly more tolerant to herbicides compared to the other weeds. These weed species; therefore, have paramount importance in competition with canola. For comparison the two means, 95% mean confidence intervals was implemented and criterion for non significant difference between tow treatments was overlapping of the confidence intervals (Table 4).

Oil Fatty Acid Profile

In 9 out of 10 cases, the effects of reduced rate of Clopyralid and Citowett mixture on canola oil composition were similar to those of control treatment. While, in comparison to control, this treatment was significantly affected the C18:1n content. Since, this unsaturated fatty acid constitute ≈ 2/3 of the oil fatty acid composition, it could be an important topic for further research and elucidation (Table 5).

Seed Viability

The germination seed rate and plumule length of canola after exposure to Clopyralid and Citowett mixture are shown in Table 6. The standard error from four replicates of 50 seeds each was less than 1% of the mean value in all cases. Results from vigor test at four-day count were unchanged at eight-days (total germination test). The standard error in plumule length was less than 3% of the mean value. Clopyralid and Citowett mixture did not significantly reduced plumule length in comparison with unexposed seed (Table 6).

Discussion:

To increase production in canola, effective weed control measures must be taken. Canola usually develops a full canopy cover at 8 weeks after emergence and can then compete well with weeds up to maturity. Little or no reduction in yield occurs if canola are kept weed free for the first 4 weeks this is the critical period of time for weed competition in canola (Gunsolus and Porter, 2004).

Canola oil comes from select rapeseed cultivars that produce low erucic acid rapeseed oil and low glucosinolate meal. Starner et al. (1999) stated that Rapeseed (*Brassica napus* and *B. rapae*) is the third most important source of vegetable oil in the world. Canola oil is considered healthy for human nutrition due to its lowest content of saturated fatty acids among vegetable oils and moderate content of poly-unsaturated fatty acids. In the present study canola oil quality was, generally unaffected by production practices investigated. The mean content of saturated fatty acids was ≈ 8.0% and was similar to those content of 7% reported in the United States (USDA, 1998) indicating that the quality of oil is comparable to that from other locations. Although fatty acid profiles vary somewhat from sample to sample, they are generally used to characterize vegetable oils from particular species or varieties of plants (Ehrensing, 2008).

Tank mixing two or more herbicides is a useful practice in intensive agriculture aiming to improve efficacy of the combined herbicides, or reduce herbicide rates and consequently to reduce the cost of weed control. In this line, Doyle and Stypa (2004) point out that much research is conducted with a new herbicide to identify doses that will maximize product value and minimize the required use rate. This view is seconded in current study in which the mixture of 2/3 Clopyralid with Citowett provided seed weight similar to those of standard treatments. Blackshaw et al. (2005) stated the benefit of integrated weed control program becomes much greater when several integration components are utilized in companion. Once an integrated weed control program has been implemented, farmers can consider choice of companion herbicides reduced rates. However, reduced rates nullify the manufacturer's guarantee of herbicide efficacy (Duchesne et al., 2004), entail concerns, and thus, should be used with great caution.

Miri and Rahimi (2009) using mixtures of herbicides stated that in Rapeseed application of post emergence herbicides mixture full rate from each − Galant Super + Lontrel − and Butisan Star at full recommended rate are good weed management treatments. In this context, the results of the present study reveled that the mixture of 2/3 full rate of Lontrel with recommended rate of Citowett is good enough for weed control similar to those of either Lontrel or Butisan Star at full recommended rate.

It could be postulated that addition of Clopyralid in a tank mix with Citowett reduced herbicide required rate substantially and improved canola weed control level. There is general agreement; herbicide application minimizing weed competition during crop development can have a significant impact on crop yields. In this line, Amin et al. (2003) stated that seed weight increases with application of some herbicide. This view is in conformity with the current research results and seconded in the average yield results taken from the trials (Table3). The average canola yield was similar to those of standard herbicides in plots treated with the tank mix combination of Clopyralid (0.7 Kg a.i. / ha) and Citowett (100 mL/ha), due to improved weed control level.

Despite what appears to be the sound basis for the use of mixtures as herbicide resistance prevention strategies, they have seldom been advocated or implemented. This is in great part due to the belief that in order to be effective, components of the mixtures must be used at the normal label rate (Diggle et al., 2003). This research, however, refuted such assumption and demonstrated that the reduced rate herbicides mixture approach is highly effective and should be researched more thoroughly in the development of any novel herbicide.

Farmers are becoming increasingly interested in more judicious weed control measures such as use of reduced rates herbicide that lower their production costs and undue environment contamination problems. The current research indicates that there is good potential to utilize reduced herbicide rates in combination with citowett. Therefore, it could be recommend this mixture as an alternative to Butisan Star and Clopyralid at recommended rates. An advantage of this mixture over the standard herbicides treatments is that it is cost effective and entails avoiding undue environmental contamination which is criterion for superiority to the treatments in question.

The distinction between live seeds and germinated seeds is important since herbicides may cause injury by retarding germination as well as destruction of germinative capacity (ISTA, 1976). Therefore, decrease in germination rate or plumule length after exposure was adequate to prove a deleterious effect of herbicide on canola's seed viability.

Together, the current study demonstrates the potential of a reduced-rate herbicides tank mixture with Citowett in canola weed management operation under field conditions. Hence, with sound recommendations for herbicides mixtures, herbicides application rates and costs could be reduced. By speculation these outcomes also may occur in other regions with similar weed population and agro-climate conditions.

Acknowledgments

The authors are grateful to vice president of research of Urmia University for financial support.

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10/15/2012

Table 1: Herbicides treatment, rate applied, and application time.

No	Herbicide treatment	Rate (Kg a.i. ha-1) or ppm in spray solution	Timing
1	Clopyralid (full rate)	0.7	Post-eme.
2	Clopyralid (2/3 of full rate)	0.47	Post-eme
3	Clopyralid + Citowett	0.47+250 ppm in spray solution	Post-eme
4	Butisan Star	3.0(full rate)	Post-eme
5	Butisan Star	2.0(2/3 of full rate)	Post-eme
6	Butisan Star+ Citowett	2.0+250 ppm in spray solution	Post-eme
7	Clopyralid + Butisan Star	0.47 + 1.0	Post-eme.
8	Clopyralid+ Butisan Star+ Citowett	0.47+1+250 ppm in spray solution	Post-eme.
9	Butisan Star + Clopyralid	2.0 + 0.23	Post-eme.
10	Butisan Star+ Clopyralid + Citowett	2.0+0.23+250 ppm in spray solution	Post-eme.
11	Weedy check		

The order of experimental treatments will be the same in table 3.

Table 2: Analysis of variance of seed yield and weeds weight of canola treated with different treatments at harvesting time.

		Mean square	
Source of variation	df	Seed yield (g)	Weed weight (g)
Block	3	53.21^{ns}	2487.47^{ns}
Treatment	10	262.60**	26580.2**
Error	30		
Total	43		

ns, and ** are non and significant at $p \leq 0.01$, respectively.

Table 3: Mean comparisons of seed yield and weeds weight in plots treated with different herbicides and associated combinations.

Treatment	Traits	
	Seed yield	Weed weight
1	28.5[a]	67.5[d]
2	10.5[cd]	114.8[bcd]
3	21.5[ab]	99.8[cd]
4	26[a]	136.8[bcd]
5	9.5[d]	147.3[bcd]
6	11.0[cd]	145.8[bcd]
7	10.75[cd]	156.3[bcd]
8	24.5[a]	254.0[ab]
9	14.75[bc]	248.5[abc]
10	12.0[cd]	273.5[a]
11	3.5[d]	309.8[a]

Numbers within a column followed by the same letters are not significantly different ($p > 0.05$).

Table 4: Average weight (g) comparisons and associated descriptive statistics of dominant weeds at harvesting time.

Treatment	Weed						
	Setaria faberil	Convolvulus arvensis	Xanthium pensylvanicum	Brassica alba	Raphnus raphanistrum	Echinchola crusgalli	Chenopodium album
Minimum	27.00	14.00	11.00	17.00	19.00	12.00	10.00
Maximum	246.00	203.00	150.00	64.00	103.00	345.00	100.00
Mean (g/m2)	69.39a	44.43b	39.46b	31.91b	40.85b	100.68a	34.52b
Std.Deviation	7.49	5.02	5.84	2.54	3.06	15.82	4.18
95% confidence limits	54.7-84.0	34.6-54.2	28.0-50.9	26.9-36-9	34.8-46.8	69.68-131.68	26.3-42.7

Means followed by the same letter in a row do not differ significantly at 5% level with Tukey's test.

Table 5: Mean comparisons of oil compositions of canola treated with a mixture of Clopyralid and Citowett at 2-leaf developmental stage with control.

Traits	Fatty acid profile (Mol %)									
	Saturated				Unsaturated					
	C16	C18	C20	C22	C16:ln7	C18:1n	C18:2n	C18:3n	C18:3n6	C18:4n3
L + C	4.12[a]	2.68[a]	1.21[a]	0.65[a]	0.18[a]	64.34[a]	18.44[a]	7.67[a]	2.03[a]	0.11[a]
Control	4.14[a]	2.72[a]	0.77[a]	0.25[a]	0.17[a]	62.38[b]	18.47[a]	5.99[a]	1.34[a]	0.08[a]
T value	-1.20	-0.33	0.15	1.54	0.03	-2.92	0.66	-0.75	-0.63	-1.26
P	0.28	0.75	0.88	0.19	0.98	0.03	0.54	0.49	0.56	0.26

L + C = treatment No. 3 (Clopyralid + Citowett).
Means followed by the same letter within columns are not significantly different (P > 0.05; Tukey's test).Fatty acid profile shows the percentage of each fatty acid component in canola. The first number in the notation at the top of each column in the profile (e.g., 18:3) indicates the number of carbon atoms in the fatty acid.

Table 6. Mean comparisons of seed germinability and plumule length of canola treated with a mixture of Clopyralid and Citowett at 2-leaf developmental stage with control.

Treatment	Viability	
	Germination rate (%) Mean ± SEM	Plumule length (mm) Mean ± SEM
Check	96.33 ± 2.20[a]	93.4 ± 0.41[a]
Clopyralid+ Citowett[•]	89.00 ± 2.64[a]	85.9 ± 2.77[a]

Means within columns with similar letters are not significantly different ($P \geq 0.05$) according to T- test. [•]Clopyralid + Citowett = Treatment No. 3.