ALLELOPATHY AND WEED CONTROL ABILITY OF THREE COVER CROPS RESIDUES, IN CONSERVATION OF AGRICULTURE

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Abstract

The weed suppressive potential of three cover crops: two forage hybrids of corn and sorghum and a local population of millet, used in conservation of agriculture_was investigated. The allelopathic potential of cover crops was studied based on bio-assays. The effect of three tested crops were used on weed dynamics (weed density, Weed Control Efficiency (WCE). Two types of residues management (TRM) were followed: standing and incorporated residues (SR, IR).Forage residues showed an allelopathic effect on germination and seedlings growth of rye-grass. *Sorghum* significantly reduced weed density. Millet residues was more efficient to control weeds whereas corn residues increased weed biomass. Weed Control Efficiency was also influenced by two types of residues management which showed a highly significant interaction with tested crops.

Introduction

Herbicide resistance of certain weeds made classical control by chemical herbicides an inefficient method. It represents also a real threat to crop production. *Lolium multiflorum* L., also called Italian rye-grass, manifested a resistance to two major herbicides, namely acetolactate synthase (ALS) and acetyl co-enzyme A carboxylase (ACCase), (Kaudun 2020). This is, in addition to the well-documented case of rigid rye-grass (*Lolium rigidum* Gaudin) resistance to the same herbicides reported by Menshari *et al.* (2016). One of the ways to deal with the complex problem of weed resistance is to find an alternative weed control tools, such as cover crop plants.

Cover crops are recognized as an effective method for weed control management. They provide early-season weed suppression like chemical and mechanical methods (Osipitan *et al.* 2018). Cover crop residues are used as mulch or incorporated into the soil delayed emergence and early growth of two weeds common chickweed (*Stellaria media* L.) and fat hen (*Chenopodium album* L.) (Kruidhof *et al.* 2009). Yellow sweet clover (*Melilotus officinalis* L.) cover controlled perennial weeds such as dandelion (*Taraxacum officinale* L.), sow thistles (*Sonchus oleraceus* L.), and annual weeds such as kochia (*Bassia scoparia* L.), flixweed (*Descurainia sophia* L.), Russian thistle (*Salsola tragus* L.), and downy brome (*Bromus tectorum* L.) (Blackshaw *et al.* 2001). Integration of sorghum (*Sorghum bicolor* L.) residues with a lower herbicide (trifluralin) rate reduced weed density without impacting broad bean (*Vicia faba* L.) yield (Alsaadawi *et al.* 2013). These cover crops expressed allelopathic activities and acted through the release of chemical substances that impact on weed development.

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With a concern to produce a clean environment, reducing the use of herbicides and the farmers expectations relating to the conservation of agriculture, choice of cover plants need to be adapted to the respective agricultural field. Thus, the present work was planned to investigate the allelopathic potential of three cover crops of forage-corn (*Zea mays* L.), forage-sorghum (*Sorghum bicolor* L.) and, millet (*Panicummiliaceum* L.). Besides the use of these cover crops, two types of residues management were also followed: Standing Residues: SR and Incorporated Residues: IR, in weed control. Corn residues and its allelopathy to weed control were reported by Jabran (2017). Allelopathy of sorghum residues in weed management was cited by Urbano *et al.* (2006). No works has been reported on the allelopathy of local Tunisian millet population. Hence, the present study was aimed to investigate the allelopathy/herbicide potential of three cover crops, and study the effectiveness of these cover crops in conventional weed management and conservation of crop plants at the field level.

Materials and Methods

The site of the experiments is located at the Higher School of Agriculture of Kef/Tunisia Experimental Station at coordinates 36° 07 '15.50" N; 8° 43 '24" E with an altitude of 524 m, in the upper semi-arid zone. The soil characteristics were alkaline sandy-clay-loamy (26 clay, 53 sand, 20% silt) texture, with Potassium (507.0 ppm), phosphorus (25.55 ppm) and organic matter content (0.95%).

Three summer crops were irrigated during the 2017/18 growing season. Two hybrids namely a 'Sancia' forage-corn hybrid (*Zea mays* L.), a 'Super Graze II' forage-sorghum hybrid [*Sorghum bicolor* (L.) Moench], and a local population of millet (*Panicum miliaceum* L.), were used to conduct the experiment. The soil preparation was followed by plowing, and re-crossings. Sowing was carried out on fallow soil by 1 precision seeder at rates of 35, 30, and 40 kg/ha, for forage-maize, forage-sorghum, and millet, respectively. The sowing was conducted on June 01, 2018. No chemical intervention (fertilization, weeding) was adopted. Irrigation was managed using well water with a dry extract of 3.5 g/l. Two types of residue management (TRM) were planned for each cereal. A management with standing residues (SR) left represents the conservation agriculture (CA) practice, via the technique of direct-drilling; and another with incorporated residues (IR) corresponds to conventional tillage practice.

At mature stage, 10 plants of each summer crop were pulled out randomly from the field. After removing the soil from roots, plant tissues were washed first by tap water then washed by distilled water and finally dried in an oven for 24 hrs at 50 °C. An estimate of dry matter content of the plant parts (leaves, stems, roots) of each plant species was carried out. From the collected tissues, plant components of each cereal were cut into 1cm pieces and stored at 5°C until extraction prepared. A fraction of 5 g of each plant component of each crop was extracted following the procedure described by Ben-Hammouda *et al.* (1995). A mixture of 80 ml of distilled water was autoclaved at 120°C for 15 min. After cooling below 50°C, 20 ml of plant (leaves, stems, roots) extracts were prepared and a pure distilled water was used as a control.

To test the allelopathic effect of plant components of three summer crops, a seedlings growth bioassay was applied. A test-species of weeds that infest fields where winter cereals are grown, which is very invasive and reported to be resistant to conventional herbicides, was considered as Italian ryegrass (*Lolium multiflorum* L.). The healthy seeds of the ryegrass were pretreated with 1.5% (v/v) sodium hypochlorite solution for 1 min to sterilize their surfaces, washed three times with distilled water for 3 min, and then dried with blotting paper. Five ml of each aqueous-extract was added to a sterile Petri dish filled with two sterile filter papers (No. 2 Whatman). Bioassays

were conducted following the procedure described by Samedani *et al.* (2013). Allelopathic effect was expressed as inhibition of germination, radicle length, and shoot length.

The inhibition of germination was calculated by the following formula:

$$\frac{\text{Control} - \text{Treatment}}{\text{Control}} \times 100$$

To evaluate the effect of crop residues of the three cover crops in the field, a count of the adventitious flora was carried out for the plots where the two TRM (SR, IR) of the 3 cover crops were installed, for the untilled fallow (UTF) and for the control with a tilled fallow (TF). After reaching the three cover crops at the panicle stage to a height of 30 cm, a glyphosate herbicide treatment was applied at a rate of 6 l/ha. Then the incorporation of residues for the IR management was done using a rotovator

Weed counting was carried out randomly on June 11, 2019 and the weed population and their above-ground biomass were recorded. The weed control efficiency (WCE) was determined as follows: WCE = (WDC - WDT) / WDC \times 100.

With, WDC: weed dry mass from the control, WDT: weed dry mass of treatments from plots grown with both TRM and tilled fallow (TF).

Bioassays were conducted in a completely randomized experimental design (CRD), with four replications. The experimental unit consisted of 2 Petri dishes. The test of the effect of cover crop species and residues management on weed control was carried out in a split-plot design with three replications. The main factor was the cover crop species (forage-corn, forage-sorghum, millet), UTF, and the control (TF), and the sub factor consisted of TRM (SR, IR). Each sub plot represented a surface of 6 m², while each main plot has a surface of 36 m². An ANOVA was done using the SAS package (SAS Institute 2002-2003). Treatments with significant effects were separated by the Fisher's LSD test (p = 0.05).

Results and Discussion

Only aqueous-extracts of the two forage species, 'Sancia' and 'Super Graze II', showed a significant effect on germination of test-weed, rye-grass (Table 1). Stems and leaves extracts of 'Sancia' showed an inhibitory effect on rye-grass germination of 57.89 and 47.37%, respectively. Extracts of 'Super Graze II' was inhibitory to rye-grass germination, and leaf-extracts showed the most inhibitory (39.29%) effect (Table 2). Only aqueous-extracts of 'Sancia' and 'Super Graze II' showed a highly significant effect in radicle growth of ryegrass (Table 1). All aqueous-extract of 'Sancia' showed an inhibitory effect in radicle growth while stems and leaves exhibited the most inhibitory effect of 57.62 and 55.52%, respectively, except stems of 'Super Graze II', all extracts were inhibitory to radicle growth. Leaf-extract was the most inhibitory (62.66%) than other extracts (Table 2) tested. The allelopathy of the two forage species was differential among plant components (Ben-Hammouda *et al.*1995, Moosavi *et al.* 2011).

Aqueous extracts of 'Sancia' showed a significant effect on the growth of the shoot of ryegrass (Table 1). Only the stem-extracts showed an inhibitory effect of 23% germination rate. Root-extracts showed slightly stimulatory to shoot growth of 7.67% (Table 2) Leaf-extracts of forage-sorghum was the most inhibitory to germination and radicle growth of rye-grass. In contrast to previous findings, stems of sorghum was reported to be the most phytotoxic (Ben-Hammouda*et al.* 1995, Moosavi *et al.* 2011).

The radicle growth of rye-grass was most pronounced than shoot growth under the action of both hybrids extracts (Tables 1 and 2). The fact confirms that radicle growth bioassay is the most reliable to detect the allelopathy promoted by crops (Motamedia *et al.* 2020).

 Table 1. Mean squares values for germination, radicle and shoot growth of rye-grass assayed with plant extracts of forage-corn ('Sancia'), forage-sorghum ('Super graze II') and, millet.

	Bioassay			
Summer cereals	Germination	Radicle growth	Shoot growth	
Forage-corn ('Sancia')	1293.75***	15.68***	2.93*	
Forage-sorghum ('Super graze II')	556.25**	13.44***	2.93 ^{NS}	
Millet	123.42 ^{NS}	0.79 ^{NS}	2.75 ^{NS}	

NS = Not significantly different at p = 0.05, *Significantly different at p = 0.05, ***Significantly different at p = 0.001.

Table 2. Effect of aqueous-extracts of forage-corn ('Sancia'), forage-sorghum ('Super graze II') and,
millet on germination (G), radicle (RG) and shoot growth (SG) of rye-grass.

		Summer crops					
		Forage con	m		Forage sorghum		
Treatments	Germination	Radicle growth	Shoot growth	Germination	Radicle growth shoot growthvalue		
Control	71.25a [†]	7.15a	6.52a	70.00a	6.83a		
Root-extracts	43.75a	5.52b	7.02a	50.00b	4.91 b		
Stem-extracts	30.00b	3.03c	5.02b	50.50b	5.86ab		
Leaf-extracts	37.50b	3.18c	5.99ab	42.50b	2.55c		
LSD $(p \leq 0.05)$	16.72	0.95	1.20	11.16	1.63		

†Means with different letters are significantly different at $p \le 0.05$, G: germination, RG: radical growth, and SG: shoot growth.

The adventitious flora of the plots, at the experimental site, was mainly composed of eight broadleaves: white buttons (*Anacyclus clavatus* Desf.), redroot pigweed (*Amaranthus retroflexus* L.), shepherd's purse (*Capsellabu rsapas-toris* L.), milk-thistle (*Silybum marianum* L.) prickly lettuce (*Lactuca seniola* L.), common mallow (*Malva sylvestris* L.), prostrate knotweed (*Polygonum aviculare* L.), and sea pink (*Limonium sinuatum* L.); and five species of grasses: great brome (*Bromus diandrus* roth.), quack grass (*Elytrigiarepens* L.), wild oat (*Avena fatua* L.), feathertop (*Pennisetum villosum* R. Br. Ex Fresen.), and rigid ryegrass. Most weed species belong to Poaceae. Some of them (white buttons, milk-thistle, wild oat, rigid ryegrass) present a high harmful effect to cereals (Melakhessou *et al.* 2020).

The species of cover crops influenced significantly weed density. The type of residues of cover crop species, had no significant effect on weed density (Table 3). The residues of forage-corn and especially those of forage-sorghum, reduced weed density of 23.39 and 45.07%, respectively. Paradoxically, the millet residues boosted weed density of 37.7% (Fig. 1). The same residues were the most efficient in controlling weeds. The present result indicates that millet residues are not a cause of reduced weed establishment, but they cause a weed growth reduction. Although type of

residues showed no significant effect on weed density, IR showed a lower density of 26.62% compared to the SR (Fig. 2). The IR (conventional) was more effective in reducing weed density than SR (conservation of crop plants). Between the two types of residues, TRM was reported to be weeding suppressive (Lemessa and Wakjira 2014). Alsaadawi *et al.* (2013) reported that the incorporation of sorghum residues resulted in weed density and biomass reductions.

Source de variation	DF	SS	MS	F-value
Block	2	0.10	0.05	0.01 ^{NS}
Crop	3	37.18	3.07	3.07^{\dagger}
Block×Crop	6	25.59	4.27	1.06 ^{NS}
TRM	1	7.94	7.94	1.96 ^{NS}
Crop×TRM	3	33.23	11.08	2.74^{NS}
Residual	8	32.33	4.04	

Table 3. ANOVA of cover crops (forage-corn, forage-sorghum, millet) effect and type of residue management (TRM).

†Significantly different at $p \le 0.09$, NS: Not significantly different at $p \le 0.05$.

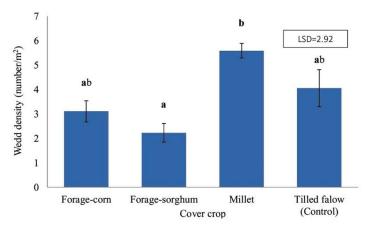


Fig. 1. Effect of cover crop residues on weed density. Error bars denote the standard error. Bars having different letters are significantly different at p≤0.05.

Cover crop showed a highly significant effect on weed control efficiency (WCE). To a lesser extent, type of residues TRM showed a significant effect on the WCE with a highly significant interaction between crop and TRM and WCE was largely influenced by cover crop species, as was the case for weed density. Millet residues were the most efficient to controlling weeds, and those from forage-sorghum were less efficient than untilled fallow (UTF). Forage-corn residues were responsible for the decrease in WCE compared to UTF (Fig. 3).Although millet residues didn't show any allelopathic effect against the tested-weed, they were the most efficient in weed control. This is probably due to the insensitivity of rye-grass at germination and seedlings stages to the allelochemicals produced by millet. Millet and corn residues expressed antagonistic effects. Millet stimulated weed density and was the most inhibitory to weed biomass (Fig. 1).

	DL	SC	СМ	F
Block	2	799.27	399.64	$3.70^{\dagger\dagger}$
Crop	3	10156.54	3385.51	31.34***
Block×Crop	6	443.86	73.98	0.68^{NS}
TRM	1	489.96	489.97	$4.54^{\dagger \dagger }$
Crop×TRM	3	7228.83	2409.61	22.31***
Residual	6	864.19	2156.08	

Table 4. ANOVA of crop	residues (forage-corn,	, forage-sorghum, n	nillet) effect and	i type of residue
management (TRM).				

***Significantly different at p = 0.0001, ^{††} Significantly different at p = 0.07, NS: Not significantly different at $p \le 0.05$.

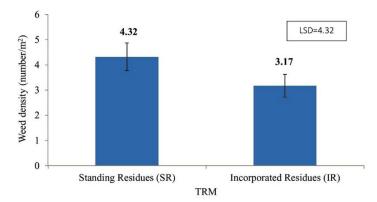


Fig. 2. Effect of type of residues management (TRM) on weed density. Error bars denote the standard error.

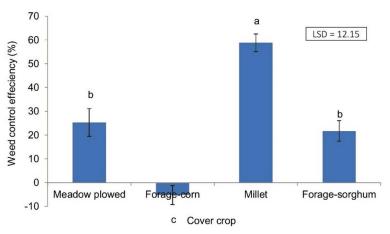


Fig. 3. Effect of cover crop residues on weed control efficiency (WCE). Error bars denote the standard error. Bars having different letters are significantly different at p≤0.05.

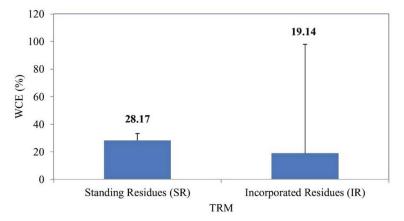


Fig. 4. Effect of type of residues management (TRM) on weed control efficiency (WCE). Error bars denote the standard error.

Unlike weed cover, SR was more efficient in weed control. SR (conservation crop plants) was more efficient than IR (conventional), in controlling weeds in first growing season. Zhang *et al.* (2021) demonstrated that a cover crop was more effective in decreasing weed density. Also weed density was more affected by incorporated residues than weed emergence. Findings of Langeroodi *et al.* (2019) showed that cover crop residues placed on the surface suppress more effectively weeds than incorporated residues. At two growing seasons of experiments, no tilling system of barley (*Hordeum vulgare* L.) and rye reduced weeds less efficiently than reduced till conventional system (Weber *et al.* 2017). Whereas Peachey *et al.* (2017) reported that cover crops non-tilled were more efficient in reducing weed emergence than conventionally planted crops with residues incorporation. This study lasted one more growing season than the previous. These contradictory effects might be due to the choice of cover crop and/or the duration of conservation practice. The incorporation of cover crop residues may influence the activity of allelochemicals released to the soil (Stegarescu *et al.* 2020).

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