Differential Autotoxicity of Five Cropped Barley Varieties

Oussama Oueslati, Faculté des Sciences de Gabès, Université de Gabès, Tunisia, **and Moncef Ben-Hammouda,** Laboratoire de Physiologie de la Production des Céréales, Ecole Supérieure d'Agriculture du Kef, Université de Jendouba, Le Kef 7100, Tunisia

ABSTRACT

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Field experiments were conducted in the North-West semi-arid zone of Tunisia during two consecutive growing seasons (2002/03, 2003/04) to study: (i) the differential auto-toxicity/allelopathy expressed by five barley (*Hordeum vulgare* L.) varieties ('Manel', 'Martin', Moumtez', 'Rihane', 'Souihli') residues (straw, stubble), used as mulch in direct drilling, (ii) the effect of soils cultivated with barley, and (iii) the impact of growing season in such auto-toxicity. The auto-toxic potential was assessed on germination and seedlings (coleoptile, radicle) growth bioassays, with 'Manel' as the test-variety. Radicle growth bioassay was the most sensitive test to detect barley auto-toxicity, when compared to coleoptile growth and germination bioassays. Barley residue-extracts showed a highly significant inhibitory effect on radicle growth of 'Manel' during the two growing seasons, with significant varietal differences. Unexpectedly, soil extracts were not active on germination and seedlings growth of barley. Stubble-extracts were more auto-toxic then those of straw, independently of variety and growing seasons. Among the five tested varieties, 'Manel' and 'Martin' were found as the most allelopathic and 'Souihli' as the least allelopathic one. Such results suggest a particular care in choosing the appropriate barley variety in monoculture under a direct drilling system. Moreover, the residues amount to be left on the soil surface must be reduced.

Keywords: Allelopathy, auto-toxicity, barley, bioassays, growing season

Barley (*Hordeum vulgare*) is a strategic cereal crop grown in Tunisia, especially in the semi-arid zone. Cereal producers continue to grow barley that is more adapted to semi-arid and rain-fed conditions which was characterized by an erratic and low rainfall and cold winter.

Corresponding author: Oussama Oueslati
Email: oussamaoueslati@yahoo.fr

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Monoculture of barley, frequently and potentially, causes a depression of grains and straw yields. This phenomenon could be partially attributed to allelopathy, and would be amplified when practicing direct drilling (7). Under conservation agriculture, abandoned biomass (mulch) on soil surface could be a source of supplementary auto-toxic substances originated by residues leachates and/or exudation that have been identified throughout barley tissues and soil extracts previously cropped with barley (5, 20). Barley plant (root, leaf, stem, seed) aqueous extracts expressed a highly auto-

toxic potential which was positively associated with three phenolic acids (p-hydroxybenzoic, syringic, p-coumaric phenolic acids) (18, 19), and varied with seasonal harvest and varieties (18). Green sugarcane (*Saccharum* spp.) post- harvest residues, expressed an allelopathic/auto-toxic potential manifested by a delay in early leaf development (25). Oat (*Avena sativa*), in a mixed culture system showed a high allelopathic potential against bread wheat (*Triticum aestivum*) (1). Both green and dry residues tissues of alfalfa (*Medicago sativa*) showed a form of auto-toxicity, reducing seedling growth, with a more pronounced effect of green tissues (13).

Despite the beneficial aspects of direct drilling (reduction of soil erosion, rebuilt of soil organic matter) yield depression may happen. Sometimes, grain yields of winter barley under direct drilling were lower than those of conventional drilling (2), and a 53% reduction in grain yield between the two types of drilling was recorded (14). Monoculture of spring barley grown with or without fertilizer under no-till, resulted in less grain yields when compared to conventional-till (15). Annual ryegrass (*Lolium multiflorum*) presents a better plant stands and produces more forage under conventional-till and no-till on shredded stubble of ryegrass and corn (*Zea mays*), when compared to no-till on ryegrass stubble (6). In an annual maize/wheat rotation, grain yield of maize was higher under permanent raised bed when compared to no-till flat system (12). Decomposing straw of wheat and oat and their associated soils were generally inhibitory to early root growth of wheat and oat, with straw being more effective (9).

This work was conducted to study the differential auto-toxic potential expressed by barley residues (straw, stubble) and soils cultivated with barley over two consecutive growing seasons.

MATERIALS AND METHODS

Plant material and field experiments.

Five barley varieties ('Manel', 'Martin', 'Moumtez', 'Rihane', 'Souihli') were grown at the Experimental Station of the *Ecole Supérieure d'Agriculture du Kef* (NW/Tunisia) during two successive growing seasons (2002/03, 2003/04). For each season, barley was sown in a fallow soil. The experimental site was located in the semi-arid zone on slightly alkaline (pH = 7.5) clay soil (10) with 48% clay, 34% sand, and 18% silt, but with only 2% organic matter**.**

For soil preparation standard, techniques are adapted in rain-fed conditions of the semi-arid zone. The field layout followed a Randomized Complete Block Design (RCBD**)** with 4-replications in six-row plots of 12 m^2 (10 m × 1.2 m) each. Seeding rate was 120 kg/ha. Climatic data relative to the two growing seasons were collected from a neighboring meteorological station (Table 1). When severe wilting was observed, plots were irrigated with 40 mm of tap water.

Preparation of aqueous extracts.

Straw and stubble of barley were randomly collected from field at mature stage. After being removed from soil, roots were washed first by tap water then with distilled water. Thereafter, they had been dried between two paper towels then chopped into 1 cm long pieces and oven dried at 50°C for 24 h. The extraction followed the procedure reported by Ben-Hammouda *et al*. (3). Barley plant parts and residues (straw, stubble) were clean and disease-free.

Table 1. Climate data* relative to the biological cycle of barley during two successive growing seasons (2002/03) and (2003/04)

Month	Rainfall (mm)		PET** (mm)		Water balance (mm)	
	2002/03	2003/04	2002/03	2003/04	2002/03	2003/04
November	117.1	13.0	61.0	141.8	56.1	-128.8
December	53.2	203.1	30.0	93.6	23.2	109.5
January	235.9	49.1	31.0	95.8	204.9	-46.7
February	66.8	16.7	51.0	136.3	15.8	-119.6
Mars	19.6	77.0	78.0	250.2	-58.4	-173.2
April	90.0	48.0	100.0	193.4	-10.0	-145.4
May	25.5	47.5	138.0	264.1	-112.5	-216.6
Total	608.1	454.4	489.0	1175.2	119.1	-720.8
Mean/month	86.9	64.9	69.9	167.9	17.0	-103.0
CV (%)	85.3	99.6	55.9	41.5	588.2	104.1

* Source: Meteorological Station of Boulifa/Kef, neighboring to the experimental site.
** Potential Evapotranspiration.

After harvest, soil samples were randomly collected from plots cultivated in 2002/03 and 2003/04 with the five barley varieties at 30 cm depth, then air dried for 1 day and sieved through 0.3 mm stitches. Soil extraction was done as follow: A sample of 250 g equivalent of dry soil was extracted in 250 ml distilled water on a horizontal shaker for 24 h at 200 rpm (22). Soil suspensions were filtered across Whatman # 2 filter paper by gravity and stored at less than 5°C until bioassay.

Bioassays of barley extracts.

Extracts of the five barley varieties were tested for auto-toxicity on germination and seedlings (radicle, coleoptile) growth using 'Manel' as a test-variety. For germination bioassays, seeds of barley were surface sterilized with 5% aqueous solution of sodium hypochlorite for 1 min, rinsed 5 times with distilled water and dried between two paper towels. Surface sterilized seeds were placed in a 10 × 150 mm Petri dish containing 15 ml of water-agar, as growth medium, amended with 20 ml of residues (straw, stubble) or soil extracts. The control was 1.2% distilled water-agar. After incubation for 35 h in dark at 25°C, seeds with the radicle extended 2 mm out of the seed coat were counted as germinated.

For seedlings growth bioassays, the growth medium was also 1.2% water-agar amended with 20 ml of residues (straw, stubble) or soil extracts. The control was 1.2% distilled water-agar. Surface-sterilization, pre-germination and bioassay setting of 'Manel' seeds, than data collection were done as reported by Ben-Hammouda *et al*. (4, 5). Inhibition of 'Manel' growth was expressed as follows: [(Control – Treatment) / Control × 100].

Statistical analysis.

Bioassays were conducted in a Complete Randomized Block Design (CRBD) with 4 replications. Data were subjected to analysis of variance (ANOVA) using SAS software (23). Treatments with a significant main effect were separated by the protected LSD

Fisher-test at the probability level of 5% (24).

The average of individual source of extracts (straw, stubble) effects was used as inhibition rate of 'Manel' radicle growth, to make a single observation relative to one variety. A combined analysis of variety effects on barley auto-toxicity across two growing seasons was conducted. The same statistical procedure was used for the average individual effect of varieties according to each source of extracts (straw, stubble). The effects of extracts from soils cultivated with barley were not considered, due to the lack of the significance test in previous analysis.

RESULTS

Germination bioassays.

During the first (2002/03) growing season, barley residues (straw, stubble) extracts of tested varieties showed a significant allelopathic/auto-toxic effect on 'Manel' (test-variety) germination (Table 2). Straw extracts of three barley varieties ('Manel', 'Martin', 'Moumtez') outof the five tested had significantly inhibited 'Manel' germination, with 'Martin' being the most phytotoxic. However, stubble-extracts of four varieties (namely 'Martin', 'Moumtez', 'Rihane', 'Souihli') showed a similar effect (Table 3). In the second (2003/04) growing season, only stubble extracts showed a significant allelopathic effect on barley germination (Table 2). Stubble extracts of three varieties ('Moumtez', 'Rihane', 'Souihli') inhibited 'Manel' germination, with 'Moumtez' extracts being the most effective. There was no stubble-extract effect on germination when 'Manel' was at the same time the test-variety and the source of extracts (Table 3). Soil-extracts effect was not significant on germination during both growing seasons (Table 2).

Coleoptile bioassays.

During the first (2002/03) growing season, only stubble-extracts manifested a highly significant effect on barley coleoptile growth (Table 2). Only 'Martin' was inhibitory to barley coleoptile growth (Table 4). However, soil extracts have no significant effect on coleoptile growth during both growing seasons

Radicle bioassays.

Extracts of barley residues (stubble, straw) showed a very highly significant effect on barley radicle growth during 2002/03 and 2003/04 (Table 2). The high significance of residues extracts is the evidence of barley auto-toxicity.

Straw extracts of the five tested barley varieties showed a differential inhibitory potential on ‘Manel’ radicle growth during the two growing seasons (2002/03, 2003/04). Extracts of ‘Moumtez’ and 'Rihane' were the most phytotoxic in all cases and extracts of ‘Souihli’ were the least effective. In the same manner was the case of stubble extracts with 'Moumtez' and ‘Rihane' as the most inhibitory in one case (2003/04), and ‘Souihli’ as the least in the two growing seasons (Table 5). However, soil-extracts effect was not significant on radicle growth during both growing seasons (Table 2). Independently of the source of plant tissues extracts, barley varieties inhibited differentially radicle growth of 'Manel' seedlings.

Table 2. Treatment mean squares for 'Manel' germination, radicle and coleoptile growth assayed with three sources of extracts (straw, stubble, soil) during 2002/03 and 2003/04 growing seasons, independently from barley variety

Growing Season	Source of extracts								
	Straw			Stubble			Soil		
	G	RL	CL	G	RL	CL	G	RL	CL
2002/03	33.02*	3.80***	0.01	190.96***	5.35***	0.13**	1.04	0.08	0.03
2003/04	104.01	4.15***	0.01	261.10**	5.05***	0.14	9.79	0.09	0.02

*, **, *** Significantly different at 5, 1 and 0.1% levels of probability, respectively. The remaining values are not significant at 5% level of probability.
G: Germination (%), RL: Radicle length (cm) and CL: Coleoptile length (cm).

Table 3. Effects of residues (straw, stubble) extracts of five barley varieties on 'Manel' germination, during 2002/03 and 2003/04 growing seasons

Growing Season	Germination (%)		
	2002/03		2003/04
Treatment	**Straw**	**Stubble**	**Stubble**
Control	92.20 a†	99.00 a†	99.00 a†
'Manel'	86.00 b	96.50 ab	96.50 ab
'Martin'	84.00 b	90.00 b	90.00 abc
'Moumtez'	86.00 b	82.50 c	78.00 d
'Rihane'	92.50 ab	92.00 b	86.50 bcd
'Souihli'	92.50 ab	82.50 c	82.50 cd
LSD $_{(5\%)}$	9.02	6.87	11.12

†Means within a column followed by different letters are significantly different at 5% level of probability.

Table 4. Effects of stubble-extracts of five barley varieties on 'Manel' coleoptile growth in 2002/03 growing season

Treatment	Coleoptile growth (cm)
Control	3.63 a†
'Manel'	3.44 a
'Martin'	3.15 b
'Moumtez'	3.38 ab
'Rihane'	3.61 a
'Souihli'	3.58 a
LSD $_{(5\%)}$	0.25

†Means within a column followed by different letters are significantly different at 5% level of probability.

Table 5. Effects of residues (straw, stubble) extracts of five barley varieties on 'Manel' radicle growth, during 2002/03 and 2003/04 growing seasons

Growing Season	Radicle growth (cm)			
	2002/03		2003/04	
Treatment	**Straw**	**Stubble**	**Straw**	**Stubble**
Control	4.39 a†	4.90 a†	4.71 a†	4.95 a†
'Manel'	2.87 b	2.04 c	2.69 bc	2.29 c
'Martin'	2.37 c	2.66 b	2.39 c	2.66 b
'Moumtez'	1.56 d	2.18 c	1.90 d	2.18 c
'Rihane'	2.05 c	1.64 d	2.05 d	1.78 d
'Souihli'	2.89 b	2.72 b	2.89 b	2.72 b
LSD $_{(5\%)}$	0.31	0.38	0.32	0.35

†Means within a column followed by different letters are significantly different at 5% level of probability.

Independently of variety and growing season, the main effect due to source of extracts was highly significant (Table 6), and stubble extracts displayed the most allelopathic effect in comparison to straw extracts (Fig. 1).

Table 6. ANOVA of growing seasons and source of barley residues (straw, stubble) extracts effects on 'Manel' radicle growth

SV	DF	SS	MS	F value	p > F
Total	**15**	**0.0301**			
Growing season (GS)	1	0.0006	0.0006	0.52	0.4828^{NS}
Source of extracts (SE)	1	0.0132	0.0132	11.10	0.0060^{**}
GS × SE	1	0.0020	0.0020	1.70	0.2168^{NS}
Error	12	0.0143	0.0011		

NS: Not significant at 5% level of probability.
** Significantly different at 1% level of probability.

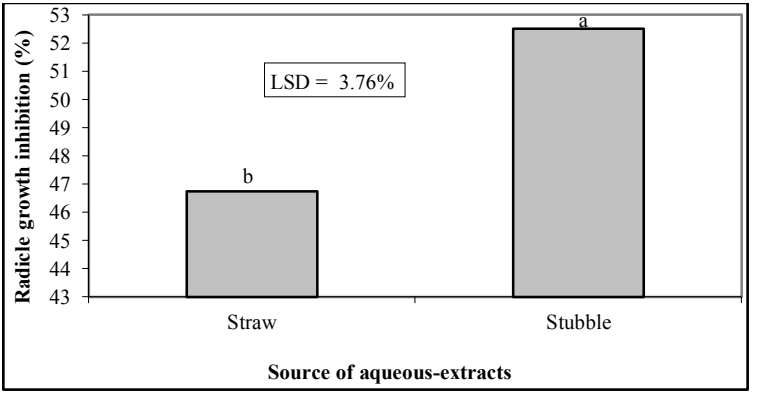

Fig. 1. Effect of source of aqueous extracts on 'Manel' radicle growth.
Bars having different letters are significantly different at 5% level of probability.

Soil-extracts did not express any significant auto-toxic potential during the two growing seasons (2002/03, 2003/04). Such result suggests that rate of allelochemicals in residues (straw, stubble) is higher and/or more effective than those of root residues or exudates released into the soil.

Barley variety × environment

The relative distribution of rainfall during the two growing seasons (2002/03, 2003/04) parallels barley radicle growth inhibition (Fig. 2 vs Table 1), though the difference is not significant (Table 7).

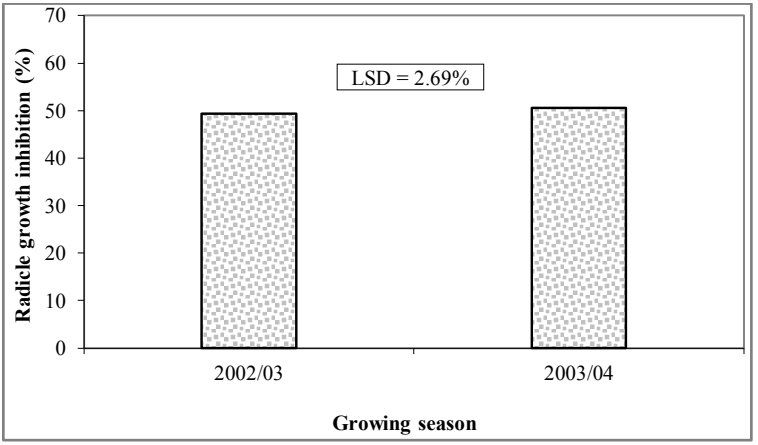

Fig. 2. Effect of growing season on 'Manel' radicle growth as a test-variety.

Table 7. ANOVA of growing season and barley variety effects on 'Manel' radicle growth

SV	DF	SS	MS	F value	p > F
Total	**39**	**0.2758**			
Growing season (GS)	1	0.0015	0.0015	0.97	0.3316^{NS}
Variety (V)	4	0.2216	0.0554	34.54	0.0001^{***}
GS × V	4	0.0045	0.0011	0.71	0.5896^{NS}
Error	30	0.0481	0.0016		

NS: Not significant at 5% level of probability.
*** Significantly different at 0.1% level of probability.

Regardless the plant source of extracts, the auto-toxicity expressed by barley varieties was not stable over the two growing seasons. Interaction between growing season and variety was not significant (Table 6). 'Manel' and 'Martin' residues extracts were the most auto-toxic when compared to 'Moumtez' and 'Rihane' ones. 'Souihli' was the least allelopathic variety (Fig. 3).

Interaction between growing season and source of extracts was not significant (Table 6), indicating that variability of allelopathic activity between source of aqueous extracts over growing season, is variety-dependant.

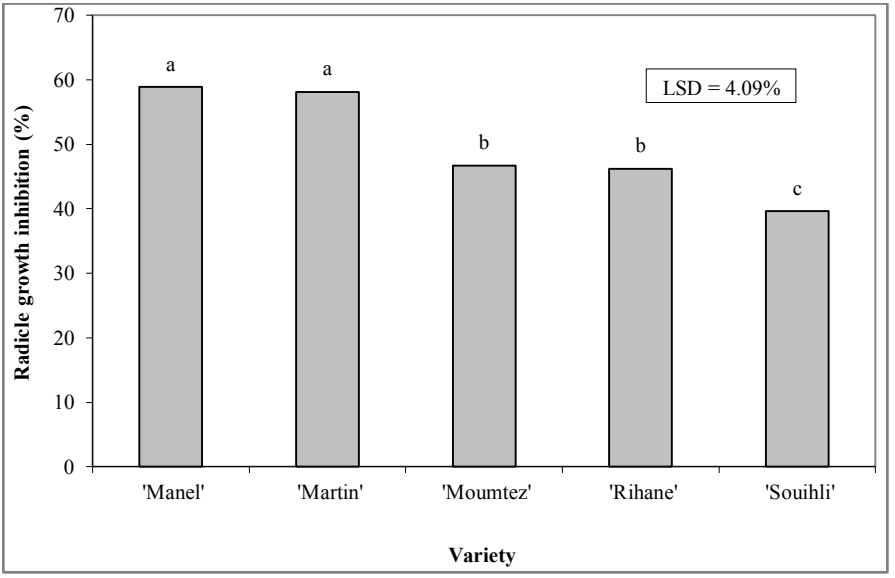

Fig. 3. Effect of variety on 'Manel' radicle growth. Bars having different letters are significantly different at 5 % level of probability.

DISCUSSION

Due to its relatively high sensitivity, only radicle growth bioassay was retained for data analysis. This procedure was supported by previous references (5, 19). There were varietal differences in inhibition of radicle growth of 'Manel'. Similar differences in allelopathic activity of barley (5, 8) and other crops (11, 17, 26) were reported. Stubble-extracts of barley were most allelopathic/auto-toxic than straw-extracts. Straw and stubble of rice expressed similar effects (16).

Soil-extracts did not significantly inhibit barley germination and growth. These results are not in agreement with the previous ones where extraction was conducted under vacuum (20). This difference could be attributed to soil extraction procedure, since extraction for this study was done by gravity that may lead to less concentrated allelochemicals.

The auto-toxicity of barley varieties was not stable over growing seasons. In addition, the interactions growing season × variety, and growing season × extracts-source were not significant. These results suggest that barely allelopathy is more inherited genetically than environmentally.

Barley auto-toxicity is variety-dependent with 'Souihli' being generally the least allelopathic variety. Choosing the crop sequence 'Manel'/'Souihli' in case of monoculture of barley could minimize the subsequent yield depression. Reducing the biomass of stubble on soil surface as mulch requires research on inhibition threshold, in order to practice properly direct drilling. Moreover, the persistence of the allelopathic effect of cereal/wheat straw that remains for more than 90 days after harvest (21) has to be considered. Laboratory experiments on auto-toxicity of extracts out of soils

cultivated with barley appeared to be insufficient to quantify its partial contribution in the overall allelopathic/auto-toxic potential. Greenhouse and field experiments may offer a better understanding.

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RESUME

Oueslati O. et Ben-Hammouda M. 2016. Autotoxicité différentielle de cinq variétés cultivées d'orge. Tunisian Journal of Plant Protection 11: 179-189.

Des essais sur champ ont été menés au Nord-Ouest de la Tunisie dans une zone semi-aride au cours de deux campagnes agricoles (2002/03, 2003/04) pour étudier: (i) l'auto-toxicité/allélopathie différentielle des résidus (paille, chaume) de l'orge (*Hordeum vulgare*), utilisés comme couverture avec le semis direct, (ii) l'effet des sols cultivés en orge, et (iii) l'impact de la saison sur l'éventuelle auto-toxicité. Cinq variétés d'orge ('Manel', 'Martin', 'Moumtez', 'Rihane', 'Souihli') ont été testées. 'Manel' a été choisie pour évaluer le potentiel auto-toxique par des bio-essais de germination et de croissance. Le bio-essai de la croissance de la radicule était le test le plus sensible pour détecter l'auto-toxicité de l'orge. Les extraits des résidus de l'orge ont montré un effet inhibiteur hautement significatif sur la croissance de la radicule de 'Manel' au cours des deux campagnes agricoles, avec des différences significatives entre les variétés. Un tel résultat suggère que la génétique est plus déterminante sur l'expression de l'auto-toxicité de l'orge que l'environnement (saison). De façon inattendue, les extraits de sol n'étaient pas actifs sur la germination et la croissance des jeunes plantes d'orge. Toutefois, indépendamment de la variété et au cours des deux campagnes agricoles, les extraits des chaumes étaient plus auto-toxiques que les extraits des pailles. Parmi les cinq variétés testées, 'Manel' et 'Martin' ont exprimé le potentiel le plus allélopathique alors que 'Souihli' a exprimé le potentiel le moins allélopathique. Les résultats indiquent que lors de la monoculture de l'orge conduite en semis direct, un soin particulier doit être porté au choix de la variété appropriée comme précédent cultural. En outre, la quantité des résidus à laisser sur le sol doit être réduite.

Most clés: Allélopathie, autotoxicité, bio-essais, orge, saison de culture

ملخص

الوسلاتي، أسامة والمنصف بنحمودة. 2016. الفوارق في السمومية الذاتية لخمسة أصناف مزروعة من الشعير.
Tunisian Journal of Plant Protection 11: 179-189.

تم إجراء تجارب ميدانية بمنطقة شبه قاحلة للشمال الغربي في تونس خلال موسمين فلاحيين (03/2002، 04/2003) و ذلك لدراسة: (1)السمومية الذاتية/المجاهضة لبقايا (قش، قصب) الشعير المستعملة كغطاء نباتي أثناء القيام بالبذر المباشر، (2) تأثير التربة المزروعة بالشعير و (3)تأثيرالظروف الموسمية على السمومية الذاتية. لذلك الغرض تم اختيار خمسة أصناف شعير ('منال'، 'مرتان'، 'ممتاز'، 'ريحان'، 'سويحلي') الأكثر تداولا في تونس لدراسة السمومية الذاتية بالاعتماد على اختبارات حيوية للإنبات ونمو البادرات، مع اختيار 'منال' كصنف اختبار. كان نمو جذير البادرات الأكثر حساسية للكشف عن سمومية الشعير، كما أظهرت مستخلصات بقايا الشعير تأثيرا مثبطا هاما على نمو جذير 'منال' خلال الموسمين المتتاليين، مع وجود فوارق معنوية بين الأصناف. تشيرهذه النتائج إلى أن الإرث الجيني هو الأكثر تأثيرا في السمومية الذاتية للشعير مقارنة بالعوامل المناخية.و بشكل غير متوقع كانت مستخلصات التربة عديمة التأثير على الإنبات ونمو بادرات الشعير، ثم بصرف النظر عن الصنف وخلال الموسمين الزراعيين كانت مستخلصات قصب الشعير أكثر سمومية من مستخلصات القش. كما كانت 'منال' و'مرتان' الأكثر سمومية و'سويحلي' الأقل سمومية من بين الأصناف

المختبرة. واستنادا على هذه النتائج يجب التدقيق في اختيار الصنف المناسب كسابق زراعي عند الزراعة الأحادية للشعير ضمن نظام البذر المباشر. وعلاوة على ذلك، يجب التقليص من كمية بقايا الشعير التي تترك على سطح الأرض.

كلمات مفتاحية: اختبار حيوية، سمومية ذاتية، شعير، مجاهضة، موسم فلاحي

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