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Revealing of resistant sources in *Cicer* species to chickpea leaf miner, *Liriomyza cicerina* (Rondani)

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Abstract The chickpea leaf miner, *Liriomyza cicerina* (Rondani) (Diptera: Agromyzidae), is an important pest of cultivated chickpea (*Cicer arietinum* L.). A 2-year field study was carried out to screen a total of 126 *Cicer* germplasm for resistance to the leaf miner during the 2012 and 2013 growing seasons. Resistance was evaluated using a visual scale of 1–9, where 1 = highly resistant and 9 = very highly susceptible under natural infestation conditions. The results showed that two *C. arietinum* accessions, ILC 3397 and Sierra, had a score of 9 on the scale, being very highly susceptible. Three germplasm, one mutant (3304) and two breeding lines (LMR 140 and LMR 160) of *C. arietinum*, were found to be highly resistant with the scores ranging from 1.5 to 2 for resistance to the leaf miner. The mutant, 3304, was detected for the first time in this study as a highly leaf miner-resistant mutant of the cultivated chickpeas while the other two breeding lines had been previously reported as highly resistant against the leaf miner. In addition, two mutants and 14 breeding lines of *C. arietinum* and two mutants and one germplasm of *C. reticulatum* were identified as resistant having the scores from 2.1 to 3 on the 1–9 scale. The results suggest that these resistant germplasm may add a new dimension to chickpea breeding programs because they possess valuable traits for resistance against the pest. The resistant chickpeas that can be grown without using pesticides are important as environmental protection and reliable food source for human health.

Keywords Chickpea · *Cicer* germplasm · Leaf miner · *Liriomyza cicerina* · Resistance

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Introduction

The cultivated chickpea, *Cicer arietinum* L. is an important food legume and the second rank after beans. It was harvested from an area of 12.7 million ha worldwide in 2017 (FAOSTAT 2018). Even if the cultivated chickpea has a yield potential of over 4000 kg per ha (Singh 1990; Singh et al. 1998), the actual yield is very low at 982 kg per ha (FAOSTAT 2018). The major reasons for the low and unstable yield in the chickpea are that the crop, like many other legumes, is grown in marginal areas and exposed to numerous biotic and abiotic stresses (Muehlbauer and Kaiser 1994,). One of the most important and influential factors among the biotic stresses is the chickpea leaf miner, *Liriomyza cicerina* (Rondani) (Diptera: Agromyzidae), in the Mediterranean basin (Reed et al. 1987; Singh and Weigand 1994; El-Bouhssini et al. 2008; Cikman and Civelek 2006).

Adult females of the leaf miner puncture both the upper and lower surfaces of leaves with their ovipositors to feed and lay their eggs. After a period of 4 days, the larvae open tunnels along the parenchyma tissue and leave secretions of whitish mines on the leaflets (Mahesh et al. 2015; Tran and Takagi 2009). The damage caused by larvae in intensive infections causes reducing the photosynthetic area which leads to falling of leaflets (Fenoglio and Salvo 2009). This damage results in significant yield losses up to 40% in chickpea (Reed et al. 1987; Cikman and Civelek 2007).

The leaf miner can be controlled using chemical insecticides, biological agents (parasitoids), cultural practices and host plant resistance (Sharma et al. 2007; Cikman et al. 2008). As much of chickpea cultivation is in marginal areas, the use of insecticides and biological agents may not be economical due to the increased unit cost. The use of resistant germplasm has been reported as the most suitable practice for the control of the leaf miner (Weigand 1990; Singh and Weigand 2006). Thus, the improvement of chickpea cultivars for resistance to the leaf miner is a major concern in integrated pest management (IPM) programs (Videla and Valladares 2007; Toker et al. 2010, 2012; Ikten et al. 2015). In previous studies, 200 annual wild *Cicer* species were screened for resistance to the leaf miner at the International Center for Agriculture Research in the Dry Areas (ICARDA) and two accessions of *C. cuneatum* Hochstetter ex A.Rich. 10 accessions of *C. judaicum* Boissier were found to be highly resistant (Singh and Weigand 1994), but *C. cuneatum* and *C. judaicum* do not have compatibility with the cultivated chickpea in interspesific crosses (Mallikarjuna and Muehlbauer 2011). Although *C. reticulatum* Ladiz. and *C. echinospermum* Davis can be crossed with the cultivated chickpea, only one *C. reticulatum* accession has been reported resistant with a score of 3 on the 1–9 visual scale (Robertson et al. 1995). Therefore, in this study, we explored the potential of genetic *resources* for finding new leaf miner resistance sources within our available collection of chickpea germplasm including some accessions, mutants and breeding lines in three *Cicer* species.

Materials and methods

Chickpea germplasm

A total of 126 chickpea germplasm in three annual *Cicer* species (*C. arietinum*, *C. reticulatum* and *C. echinospermum*) were screened for resistance to the leaf miner (Table 1). The FLIP (Food Legume Improvement Program) and LMR (Leaf Miner Resistant) breeding lines and the accession, ILC 3397 were provided by ICARDA. The mutant chickpeas used in the study were developed by irradiation of 200, 300 and 400 Gy gamma rays to the seeds of *C. arietinum* and *C. reticulatum* species at the Turkish Atomic Energy Agency (TAEK), Ankara, Turkey. Five hundred seeds from each of 15 chickpea germplasm belonging to three *Cicer* species were treated and generated from M_1 to M_5 (Toker et al. 2005, 2014). The numbers in the mutant names of *C. arietinum* species indicate cultivar name, irradiation dose and mutant number; for instance, in the name of 3325, the first 3 represent cultivar name, the second 3 irradiation dose of 300 Gy and 25 mutant number.

Cultivation of chickpea germplasm

The experiments were conducted in two consecutive seasons (2012 and 2013) in randomized block design with two replicates at the experimental fields of Akdeniz University, Antalya, Turkey (30°44′E, 36°52′N and 51 m from sea level). In each replicate, each germplasm was represented one row including 20 plants. The plot size was 2 × 2 m with 2 rows each two meter length and with distance between rows 45 cm. All the germplasm were sown by hand at a uniform depth of 5 cm in February each year. Weed control was done by hand at both seedling stage and before flowering. Fertilization with N, P and K was made at a rate of 15 kg per ha prior to sowing.

Data collection and evalution

Incidence of leaf miner was evaluated using a 1–9 visual scale, developed by Singh and Weigand (1994) and modified by Toker et al. (2010), under natural insect infestation in the field (Table 2). According to the scale, the germplasm with a rate between 1 and 4 were resistant, those with 5 were tolerant, and those having a rate between 6 and 9 were susceptible. Data related to incidence of leaf miner were collected from all the plants in each row at three different stages of plant growth including seedling, flowering and mid-podding stages. The highest score in the three stages was used for evaluation.

In addition to the insect incidence score, the following morphological and agronomical data were recorded on 10 mature plants selected from each germplasm just

Table 1 Chickpea germplasm belonging to three *Cicer* species screened for resistance to leaf miner

Cicer arietinum	Origin/Donor Institute	*Cicer arietinum*	Origin/Donor Institute	*Cicer reticulatum*	Origin/Donor Institute	*Cicer reticulatum*	Origin/Donor Institute	*Cicer echinospermum*	Origin/Donor Institute
ILC 3397	ICARDA	LMR 196	ICARDA	AWC 602–8	AU	AWC 608–1	AU	C. *echinospermum*-1	AU
Sierra	USDA	LMR 156	ICARDA	TR 135	AARI	AWC 610	AU	AWC 305	AU
3325 (M)	AU	FLIP 2005-6C	ICARDA	AWC 621	AU	TR 39221-S	AARI	AWC 303	AU
31,160	AU	LMR 173	ICARDA	AWC 625	AU	AWC 627	AU	AWC 306	AU
TUR 1702	AARI	LMR 202	ICARDA	TR 13	AU	TR 58079	AU	AWC 307	AU
TUR 01124	AARI	LMR 146	ICARDA	AWC 602	AU	AWC 605	AU	AWC 304	AU
5401 (M)	AU	LMR 149	ICARDA	AWC 602–3	AU	C. ret-1	AU	AWC 304–1	AU
CA 2969	CSIC	FLIP 2005-3C	ICARDA	AWC 602–4	AU	AWC 601	AU		
ICC 4951 (M)	ICRISAT	LMR 144	ICARDA	AWC 609	AU	TR 58079–1	AARI		
ICC 6119 (M)	ICRISAT	LMR 150	ICARDA	AWC 611–1 (M)	AU	AWC 602–6	AU		
YAR	AU	LMR 155	ICARDA	AWC 602–5	AU	TR 39221–9-1	AARI		
1302 (M)	AU	31,109	ICARDA	AWC 600–2	AU	AWC 614	AU		
ICC 4951 (M)	AU	LMR 138	ICARDA	AWC 600	AU	TR 39221–2	AU		
TUR 03066	AARI	3205	ICARDA	C. ret. (M)	AU	AWC 612	AU		
31,126	AU	LMR 125	ICARDA	TR 39221–12	AARI	AWC 609–2	AU		
31,145	AU	FLIP 2005-4C	ICARDA	AWC 611–2 (M)	AU	AWC 611–3 (M)	AU		
TUR 01680	AARI	FLIP 2005-5C	ICARDA	C. ret. -1 (M)	AU	C. ret. Control	AU		
31,133	AU	LMR 139	ICARDA	TR 23039	AARI	TR-39221-1	AARI		
31,141	AU	LMR 153	ICARDA	TR 39221	AARI	AWC 610	AU		
TUR 1469	AARI	LMR 29	ICARDA	AWC 605–1	AU	AWC 603	AU		
5305 B (M)	AU	LMR 159	ICARDA	AWC 632	AU	AWC 609–3	AU		
31,124	AU	LMR 158	ICARDA	AWC 633	AU	AWC 610–1	AU		
31,138	AU	3404–2 (M)	ICARDA	AWC 602–7	AU	AWC 608	AU		
31,151	AU	LMR 164	ICARDA	AWC 608–2	AU	AWC 612 B (M)	AU		
31,132	AU	LMR 124	ICARDA	AWC 602–9	AU	AWC 612–3 (M)	AU		
LMR 134	ICARDA	LMR 135	ICARDA	AWC 602–1	AU	AWC 623	AU		
LMR 186	ICARDA	LMR 40	ICARDA	AWC 612–1	AU				
ICC 6119	ICRISAT	LMR 81	ICARDA	C. ret. (M) WF	AU				
LMR 133	ICARDA	LMR 140	ICARDA	AWC 600–1	AU				

Table 1 (continued)

Cicer arietinum	Origin/Donor Institute	*Cicer arietinum*	Origin/Donor Institute	*Cicer reticulatum*	Origin/Donor Institute	*Cicer reticulatum*	Origin/Donor Institute	*Cicer echinospermum*	Origin/Donor Institute
LMR 123	ICARDA	LMR 160	ICARDA	AWC 609–1	AU				
LMR 151	ICARDA	3304 (M)	ICARDA	AWC 609–4	AU				

M in parenthesis indicates that the germplasm is a mutant of relating species

ICARDA International Center for Agriculture Research in the Dry Areas (Aleppo, Syria), *USDA* The United States Department of Agriculture (Washington, DC.), *AU* Akdeniz University (Antalya, Turkey), *AARI* Aegean Agricultural Research Institute (Menemen, İzmir, Turkey), *CSIC* Institute for Sustainable Agriculture (Cordoba, Spain), *ICRISAT* International Crops Research Institute for the Semi-Arid Tropics (Hyderabad, India)

Table 2 A quantitative 1–9 scale for leaf miner resistance of chickpea germplasm

Resistance rating	Reaction category	Appearance of germplasm
1	Very highly resistant	Free from any damage
2	Highly resistant	A few mines evident after careful observation
3	Resistant	A few mines in less than 20% of the leaflets, no defoliation
4	Moderately resistant	Mines present in 21 to 30% of the leaflets, no defoliation
5	Tolerant	Mines present in 31 to 40% of the leaflets, some defoliation in the lower half of plants
6	Moderately susceptible	Many mines in 41 to 50% of the leaflets, defoliation of 10% of the lower leaflets
7	Susceptible	Many mines in 51 to 70% of the leaflets, defoliation of 10 to 20% of the lower and upper leaflets
8	Highly susceptible	Many mines in 70 to 90% of the leaflets, defoliation of 20 to 30% of the lower and upper leaflets
9	Very highly susceptible	Many mines in almost all of the leaflets (90%) and defoliation greater than 31%

before and after harvest. These are canopy width (cm), plant height (cm), first pod set (cm), number of pods and stem per plant (no), biological and seed yields (g) and 100-seed weight (g).

Soil properties

Soil samples were taken from the experimental field at a depth of 0–30 cm, and then analyzed for organic matter, nitrogen (N), soil texture, pH, calcium carbonate ($CaCO_3$), phosphorus (P), potassium (K), calcium (Ca), sodium (Na), iron (Fe) and zinc (Zn). Organic matter and N in the soil were at low levels, and the soil texture was sandy-clay-loam with a pH value of 7.69. $CaCO_3$ was 26.5%, and the electrical conductivity was 0.93 mS/cm. Most of the nutritional elements were balanced, while Fe and Zn were thought to be deficient due to the high pH.

Weather conditions

Since higher temperature, humidity and rainfall had a positive impact on population development of chickpea leaf miner (Cikman and Civelek 2006), environmental conditions in the experimental area were recorded throughout the study. The weather in the study area was characteristically warm, and rainfall was irregular, typical of a Mediterranean climate. As temperature increased gradually during the spring months, rainfall reduced remarkably during the same period. The total rainfall was 890 and 925 mm in 2012 and 2013 growing seasons, respectively. Rainfall was irregular in the second year. Minimum and maximum temperatures were 3.6 °C and 41.6 °C in 2012, 1.9 °C and 43.1 °C in 2013, respectively.

Data analyses

The scores of the germplasm for resistance to the leaf miner were converted from numerical data (1–9 scale) to percentages (%) for analysis of variance (ANOVA) using GLM function under the packet program of SPSS 24.0 (IBM Corp 2013). Duncan's multiple range test (DMRT) was used to test for differences among the germplasm means at 5% level ($P \leq 0.05$) of probability. Correlation analysis in MINITAB-17 software was used to determine correlation coefficient between different agro-morphological characteristics with leaf miner.

Results

Significant differences were found among the *Cicer* species in terms of incidence of leaf miner ($F = 30.159$; df = 124; $P \leq 0.05$). Considering the leaf miner incidence scores of the 126 germplasm tested, none of them was completely resistant to the leaf miner in both years. Three germplasm, one mutant (3304) and two breeding lines (LMR 140 and LMR 160) of *C. arietinum*, were found to be highly resistant with the scores ranging from 1.5 to 2 for resistance to the leaf miner (Table 3). Nineteen germplasm, two mutants and fourteen breeding lines of *C. arietinum* and two mutants and one accession of *C. reticulatum*, were resistant having the scores from 2.1 to 3. A total of 25 germplasm, 16 lines and two accessions of *C. arietinum* and seven accessions of *C. reticulatum*, were determined as moderately resistant with the scores changing between 3.1 and 4. Twenty-four germplasm, four accessions of *C. arietinum*, and one mutant and 14 accessions of *C. reticulatum* and five accessions of *C. echinospermum*, had the scores between 4.1 and 5 and were categorized as tolerant.

As for the susceptible germplasm, a total of 20 germplasm, one mutant and one accession of *C. arietinum*, two mutants and 14 accessions of *C. reticulatum* and two accessions of *C. echinospermum*, were determined as moderately susceptible with the scores ranging from 5.1 to 6 (Table 3). Nineteen germplasm, two mutants and six accessions of *C. arietinum* and three mutants and eight accessions of *C. reticulatum*, were categorized as susceptible with varying scores between 6.1 and 7. Thirteen germplasm, three mutants and five accessions of *C. arietinum* and five accessions of *C. reticulatum*, were highly susceptible with the scores between 7.1 and 8. Lastly, three germplasm, one mutant and two accessions of *C. arietinum*, were highly susceptible with the scores ranging from 8.1 to 9. Two of the last three germplasm (Sierra and ILC 3397) had a score of 9 in both years, which indicated that natural infestation of the leaf miner had occurred in both years.

The minimum and maximum values of agro-morphological characteristics and chlorophyll content in the germplasm of *Cicer* species were as follows; plant height 3–59 cm, number of stems per plant 1–118.4, first pod height 1–35 cm, canopy width 9–78 cm, number of pods per plant 3.5–355, biological yield 10–890 g, seed yield 0.4–351.5 g and 100-seed weight 7.8–49.4 g (Table 4). Susceptibility to the leaf miner was negatively significantly correlated with biological yield, seed yield, first pod set and 100-seed weight (Table 4).

Discussion

The results of the present study showed that one mutant (3304) and two breeding lines (LMR 140 and LMR 160) of *C. arietinum* were highly resistant to the leaf miner with the scores between 1.5 and 2 on the 1–9 scale (Table 3). The mutant (3304) exhibited the same level of resistance to the leaf miner as LMR 140 and LMR 160 which had previously been developed as leaf miner-resistant lines at ICARDA. Prior to this study, germplasm resources of the cultivated chickpea had already been screened for resistance to the leaf miner (Reed et al. 1987). From this previous study, 21 out of 9500 germplasm were assessed as resistant and three were released for cultivation. In another study, none of the 7000 germplasm tested at ICARDA was found to be highly

Table 3 Numerical distribution of 126 germplasm belonging to three *Cicer* spp. for resistance to leaf miner based on their scores in the 1–9 visual scale

Cicer spp.	Germplasm	Visual scale for resistance against leaf miner								
		Very highly resistant (1)	Highly resistant (1.1–2)	Resistant (2.1–3)	Moderately resistant (3.1–4)	Tolerant (4.1–5)	Moderately susceptible (5.1–6)	Susceptible (6.1–7)	Highly susceptible (7.1–8)	Very highly susceptible (8.1–9)
C. arietinum	Accession				2	4	1	6	5	2
	Lines		2	14	16					
	Mutants		1	2			1	2	3	1
C. reticulatum	Accession			1	7	14	14	8	5	
	Mutants			2		1	2	3		
C. echinospermum	Accession					5	2			
	Mutants									

resistant to the leaf miner (Singh and Weigand 1994). Malhotra et al. (2007) reported that seven breeding lines were resistant to the leaf miner in resistance studies. Toker et al. (2010) found a relationship between chickpea leaf shape and leaf miner resistance, i.e.; genotypes with multipinnate leaf shapes were reported to have structural resistance. Similarly, Singh and Weigand (2006) reported three resistant germplasm resources in the "kabuli" chickpea (ILC 3800, ILC 5901, and ILC 7738), which had a multipinnate leaf shape. A "desi" chickpea germplasm, ICC 6119, was also detected as resistant (Toker et al. 2012).

As alternative genetic resources, Singh and Weigand (1994) reported resistant resources in wild *Cicer* species for the leaf miner after screening 200 germplasm of *Cicer* species. According to their results, two accessions (ILWC 40 and ILWC 187) of *C. cuneatum* Hochst. ex Rich. and 10 accessions (ILWC 44, ILWC 46, ILWC 56, ILWC 57, ILWC 58, ILWC 95, ILWC 103, ILWC 196, ILWC 2026 and ILWC 207) of *C. judaicum* Boiss. were found to be highly resistant to the leaf miner with the score of 2 on the 1–9 scale. A total of 23 accessions, 18 from *C. judaicum*, 4 from *C. pinnatifidum* Jaub. & Spach. and 1 from *C. reticulatum* were recognized as resistant with a score of 3 on the scale. Available and additional resistant resources in *Cicer* species were screened for resistance to the leaf miner since none of the germplasm were reported as very highly resistant. Ikten et al. (2015) introduced a mutant in *C. reticulatum*, highly resistant to the leaf miner after screening 20 germplasm of *Cicer* species. This mutant line of *C. reticulatum* has been registered as an alternative genetic resource. In the current study, two mutants (3205 and 3404) and 14 breeding lines (LMR 40, LMR 81, LMR 135, LMR 124, LMR 164, LMR 158, LMR 159, LMR 29, LMR 153, LMR 139, FLIP 2005-4C, FLIP 2005-5C, LMR 125 and LMR 138) of *C. arietinum* and two mutants (AWC 612 B and AWC 612–3) and one accession (AWC 623) of *C. reticulatum*, were identified as resistant having the scores from 2.1 to 3 on the 1–9 scale (Table 3). While the genus *Cicer* L. consists of 49 taxa (Smykal et al. 2015), *C. reticulatum* and *C. echinospermum* can be hybridized with cultivated chickpeas (Ladizinsky and Adler 1976; Singh et al. 2005). Some germplasm of *C. echinospermum* and *C. reticulatum* cannot only be crossed with the cultivated chickpea (Adak et al. 2017; Koseoglu et al.

Table 4 Means, minimum-maximum values and correlation of leaf miner susceptibility (LMS) with each agro-morphological characteristic of *Cicer* species tested in the present investigation ($n = 126$)

Characteristics	*C. arietinum*			*C. reticulatum*			*C. echinospermum*			Correlation with LMS
	$\bar{x} \pm S_{\bar{x}}$	Min	Max	$\bar{x} \pm S_{\bar{x}}$	Min	Max	$\bar{x} \pm S_{\bar{x}}$	Min	Max	
Plant height (cm)	43.8 ± 0.6	18.0	59.0	28.1 ± 0.8	3.0	50.0	14.5 ± 1.1	7.0	21.5	−0.007
Stem number (no)	1.8 ± 0.1	1.0	15.0	4.8 ± 0.7	1.0	118.4	6.7 ± 0.5	4.0	9.5	−0.118
First pod set (cm)	20.3 ± 0.5	6.5	35.0	7.5 ± 0.4	1.0	35.0	3.6 ± 0.3	2.0	6.5	−0.194**
Canopy width (cm)	25.0 ± 0.7	9.0	72.0	43.9 ± 1.1	10.0	78.0	32.3 ± 2.5	18.0	45.5	−0.101
Pod number (no)	27.7 ± 1.2	5.0	108.5	36.3 ± 2.5	4.5	355.0	15.6 ± 2.3	3.5	36.0	−0.139
Biological yield (g)	299.8 ± 13.0	15.0	890.0	113.2 ± 5.3	15.0	445.0	37.9 ± 4.4	10	70.0	−0.414**
Seed yield (g)	121.9 ± 5.4	0.4	351.5	37.0 ± 2.2	0.9	145.0	6.3 ± 1.3	1.0	13.4	−0.397**
100-Seed weight (g)	29.3 ± 0.7	9.2	49.4	17.9 ± 0.4	7.8	45.5	10.6 ± 0.4	8.7	14.2	−0.215**

**Correlation is significant at the 0.01 level

2017; Kahraman et al. 2017), but some ones of the species have also been reported to have agro-morphological characteristics to improve the chickpea and they were resistant to biotic and abiotic stresses (Talip et al. 2018).

The leaf miner susceptibility was negatively significantly correlated with biological yield, seed yield, first pod set and 100-seed weight (Table 4). This indicated that the resistant germplasm had more seed and biological yields, more pods per plant, and more 100-seed weight. Similar relationships were determined between the leaf miner resistance and leaflet size by Toker et al. (2010). As in the current study, it was reported that small leaflet size was not preferred by the insect (Toker et al. 2010). Insect resistance in legumes was divided into three categories consisting of (i) structural defenses, (ii) secondary metabolites and (iii) anti-nutritional compounds (Edwards and Singh 2006). As a good samples of secondary metabolites, chickpea exudates some organic acids including citric, malic, oxalic, quinic and succinic acids on all green parts of surface (Rembold 1981; Khanna-Chopra and Sinha 1987; Toker et al. 2004) providing resistance to insect in chickpea (Rembold 1981; Khanna-Chopra and Sinha 1987). Resistance in the present study could be both due to structural defenses and secondary metabolites. Further studies are needed to clarify the role of plant secondary metabolites on the resistance of the studied germplasms.

The "desi" chickpea germplasm, ICC 6119 cotegorized as moderately resistant with a score of 4, suffered from transiet Fe-deficiency chlorosis during the seedling stage since Fe was deficient in the experimental area due to the high pH. So, during the seedling stage, ICC 6119 was not chosen by the insect which may have been due to the pale yellow tissues of the plant. This germplasm was preferred by the insect when it became green. The same was also observed by Toker et al. (2012).

In conclusion, in this work we identified several chickpea germplasms showing high to moderate resistant to the leaf miner. These genetic materials can be used in breeding programs and can be tested in the field for growing under IPM without the use of pesticides. Chickpea germplasms that can be grown without pesticides are ecological friendly and ensure environmental sustainability, allow increase agricultural productivity and guarantees food safety for human health.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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