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Contents

Essential oils rich in pulegone for insecticide purpose against legume bruchus species: Case of Ziziphora hispanica L. and Mentha pulegium L.

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A B S T R A C T

Ziziphora hispanica L. and Mentha pulegium L. are aromatic shrubs known to produce essential oils demanded by food industries. This study reports on the insecticidal activity of these two oils harvested in Khenifra (Morocco) and of its major compound (Pulegone) against the main legume bruchids. Chromatographic analysis revealed the presence of 33 compounds in the essential oil of Ziziphora hispanica (EOZH) and 24 compounds in the essential oil of Mentha pulegium (EOMP), with pulegone being the most dominant in both with percentages respectively of 79.1% and 63.8%. According to the insecticidal activities exerted, the two essential oils, as well as the pulegone, showed significant results against all the bruchids tested of legumes. Pulegone has shown good insecticidal activity, thus the lethal doses of 50% are between 61.6mg/g and 74.4mg/g, which explains the remarkable activity of the two essential oils which are rich in this molecule. moreover, the addition of essential oils in the storage of legumes does not have a significant effect on the legumes and does not diminish their nutritional qualities. This study indicates that essential oils and their main constituent have the potential to be developed into botanical insecticides.

Keywords: essential oils; pulegone; insecticidal activities; bruchids; legumes

1. Introduction

Grain legumes are abundant in proteins, oligosaccharides, slow-digesting starches, dietary fiber and phenolic content, with just a minor quantity of lipids [1,2]. These elements could contribute to the nutrition advantages, such as their effects on chronic diseases due in particular resulting from deficiencies in biochemical elements [3].

Legumes storage loss assessment and mitigation strategies have occupied a prominent position on the scientific research agenda during the past few decades, but farmers in underdeveloped countries continue to suffer enormous losses despite the attention the framed governments have given to these issues [4–6]. Adults of certain insect species such as Bruchidae beetles (Coleoptera: Bruchidae) attack legume grains and cause serious damage, reducing the quality and quantity of stored biomass [7].

Bruchus lentis Frölich, Bruchus pisorum L., Bruchus rufimanus Boheman are an important pest on lentils, peas and faba beans respectively, and is now distributed in all countries of the world, particularly North Africa [8–10].

Several options have proved effective in controlling storage this insect pests, including environmental manipulations to discourage their growth, development and reproduction [11,12]. Such experimental options can be intervened by employing a number of control measures such as the use of chemical insecticides, biological and physical control methods or a combination of these operations [13–15]. Chemical pesticides work well against pests of storage insects, but they are also always accompanied by a number of disadvantages, including high prices of this products and worries about environmental contamination and food safety [16].

Previous studies pointed out the possibility of an integrated pest insects management strategy by using other constituents such as essential oils to control bruchids development in food legume stocks [17–19]. In these regards, many studies reported the efficacy of various plant species essential oils for the control of various beetles. These natural products are extracted from plants using traditional and innovative techniques [20]. Their excellent efficiency against a variety of pests, numerous modes of action, low toxicity of residues after application, and relatively inexpensive production procedure make them a potential and suitable option [21–23].

In this study, we investigated the insecticidal effects of the two essential oils of Ziziphora hispanica (EOZH) and Mentha pulegium (EOMP) against the main legume bruchids such as: Bruchus lentis, Bruchus pisorum, Bruchus rufimanus. The two essential oils and its main constituent, the pulegone, were evaluated by several tests, in order to determine certain parameters to know the effects of the compounds tested on the nutritional values.

2. Materials and methods

2.1. Essential oils: Extraction and analysis

The essential oils used in this study were extracted from aerial parts of both aromatic plants: Ziziphora hispanica L. and Mentha pulegium L. Plant materials collected from Morocco (Midle Atlas), were air-dried at room temperature (20–25 °C) and under the dark for one month and then stored in glass boxes. The species was identified by ESTK-USMS team and it is classified in the herbarium with the accession numbers: ZH4-2021 and MP7-2021. Essential oils were extracted by hydro-distillation of the dried plant leaves (250 g of each sample in 1L of distilled water) using a Clevenger-type apparatus for 3 h.

Essential oils were characterised by GC-MS, whose apparatus operating conditions were as follows: A Shimadzu 2010 with injector temperature 200 °C; detector interface at 250 °C; ion source 200; carrier gas helium; OV-1 column (non-polar, WCOT); Column flow: 1 ml/min; column ramp: 60 °C (no hold), 5 °C per min then held at 280 °C for 5 min. The all compounds were identified by comparing they mass spectra and retention indices (derived relative to n-alkanes) with the National Institute of Standards and Technology (NIST) collection [24].

2.2. Insecticidal assay

Bruchus adults were obtained from the breeding stock maintained at the laboratory of EST-Khenifra (University Sultan Moulay Slimane) at 28 ± 2 °C in glass containers containing leguminous and closed with non-woven fabric (Bruchus lentis in lentils, Bruchus pisorum in peas and Bruchus rufimanus in faba bean). The insecticidal assay of essential oils and the pure pulegone molecule consisted of an adaptation of the method proposed by several previous works [25–27]. For each bioassay, a weight in mg of each sample was added to 2.0 g of the leguminous biomass of each species, and the mixture was grinded for 5 min in order to obtain a final powder.

Next, five aliquots of 200 mg of the powder were placed in a Petri plate (90 × 100 mm), which was then incubated at 25 °C for 2 h. After this period, 10 insects were transferred to the plate, and the bioassay was maintained in the dark at 28 ± 2 °C. Each assay was performed in triplicate, and the weight of leguminous and insects was determined at time zero and after 7 days. Mortality rates (%) were recorded after 7 days of experiment. The final concentrations of all samples in the disks were 20–400 mg/g (mg per g of leguminous). The determination of the lethal doses of 50% (LD50) are determined by linear interpolation in curves giving the percentage of mortality as a function of the logarithm of the concentrations tested.

2.3. Nutritional Indices

Four nutritional indices, including Feeding-Deterrence Index (FDI), Relative Consumption Rate (RCR), Relative Biomass Gain Rate (RBGR), and Efficiency of Conversion of Ingested food (ECIF) were calculated respectively on the basis of formulas [28,29]:

$$FDI(\%) = \frac{A-B}{A} \times 100 \tag{1}$$

$$RCR(mg.mg^{-1}.day^{-1}) = \frac{C}{(D \times days)} \tag{2}$$

$$RBGR(mg.mg^{-1}.day^{-1}) = \frac{E}{(D \times days)} \tag{3}$$

$$ECIF(\%) = \frac{E}{C} \times 100 \tag{4}$$

where:
A is the mass of food ingested by insects in the control;
B is the mass of food ingested by insects in the test;
C is the mass (mg) of ingested food;
D corresponds to the initial insect biomass (mg);
E corresponds to the biomass gained (mg).

According to the FDI, the samples were classified as: no-deterrent (FDI < 20%), weakly deterrent (50% > FDI ≥ 20%), moderately deterrent (70% > FDI ≥ 50%), or strongly deterrent (FDI ≥ 70%).

2.4. Evaluation of major biochemical constituents

After storing of 50 g of leguminous grains for a period of three months with a concentration of 100mg/g of each essential oil and pulegone, the different major biochemical components of leguminous, such as proteins, carbohydrates, and total fat, were measured using standards: ISO 26642:2010, ISO 16634-2:2016 and 11085:2015, respectively [30–32].

2.5. Statistical analysis

LD50s, Nutritional parameters and nutritional values of leguminous are expressed as mean of three tests with uncertainty at significance level (alpha) = 5 %. Analysis of variance (ANOVA) followed by Tukey's test was employed to compare the means of LD50 of each product (Essential oils and Pulegone) and nutritional values of leguminous (lipids proteins and sugars). ANOVA was performed to check the significant relationship between treatment sets in various bioassays [33].

3. Results

After obtaining the two essential oils EOZH and EOMP by hydrodistillation, quantitative analyses were made by the GC-MS technique. The qualitative chromatograms for GC-MS analyses of the two essential oils are reported in the Figures 1 and 2. The analysis data are mentioned in Table 1.

The chemical compositions of the two essential oils reveal the presence of pulegone as a major molecule with a percentage of 79.1% for EOZH and 63.8% for EOMP.

In addition, these results accompanied by the presence of other compounds with moderate percentages, we note the presence of limonene (1-methyl-4-(1-methylethenyl)-cyclohexene) (3.5%) and 4-Menthen-8-ol (2-methyl-2-(4-methylcyclohexen-1-yl)propan-1-ol) (2.8%) in EOZH, and of the presence of menthone ((2S,5R)-5-methyl-2-propan-2-ylcyclohexan-1-one) 9.1%), limonene (1-methyl-4-(1-methylethenyl)-cyclohexene) (5.4%), germacrene-D ((1E,6E,8S)-1-methyl-5-methylidene-8-propan-2-ylcyclodeca-1,6-diene) (4.1%), p-menthane-3-ol (2-methyl-2-(4-methylcyclohexen-1-yl)propan-1-ol) (2.1%) and (E)-β-caryophyllene ((1R,4E,9S)-4,11,11-trimethyl-8-methylidenebicyclo[7.2.0]undec-4-ene) (2.1%) in EOMP. Other compounds were detected with different percentages and in a quantity lower than 2%, as well as some in common between the two essential oils studied.

Four components accounting about 1–1.5% were found only in EOZH: carvacrol (2-methyl-5-propan-2-ylphenol) was 1.6%; thymol (5-methyl-2-propan-2-ylphenol) was 0.9%; menthofuran (3,6-dimethyl-4,5,6,7-tetrahydro-1-benzofuran) was 0.9%; trans-isopulegone (5-methyl-2-prop-1-en-2-ylcyclohexan-1-one) was 0.8%.

Several previous works have been done on the essential oils of these two species and which confirm the present identification, among these studies, we note the research of Bekhechi et al. [34]and research by Stoyanova et al. [35] who show the presence of pulegone as the main molecule respectively in the essential oils of Ziziphora hispanica and Mentha pulegium.

The results of the insecticidal activity of the two essential oils and of pulegone against the three species of bruchids: Bruchus lentis, Bruchus pisorum and Bruchus rufimanus are given in Table 2.

These results are expressed by the lethal dose of 50% (LD50) in mg/g. the reading of the data shows the interesting activity of the products tested, particularly of the molecule of pulegone, moreover all the results are significantly different after the statistical test of ANOVA, from where, the activities noticed in the two essential oils returned to the presence of pulegone with high concentrations. EOZH always was more effective with respect to EOMP.

In fact, the LD50 against Bruchus lentis was 121.4 mg/g for EOZH and 148.1 mg/g for EOMP. Similarly, the LD50 against Bruchus pisorum was 135.5 mg/g for EOZH and 163.4 mg/g for EOMP. The same prevalent effect was found against Bruchus rufimanus for which the LD50 was 133.6 mg/g by EOZH and 137.9 mg/g by EOMP (Table 2).

Other parameters were studied to complete the insecticide study, these are the determination of Feeding-Deterrence Index (FDI), Relative Consumption Rate (RCR), Relative Biomass Gain Rate (RBGR), and Efficiency of Conversion of Ingested food (ECIF). The values obtained are displayed in Figures 1, 2, 3 and 4.

The results obtained confirm the previous results, so the two essential oils EOZH and EOMP, and the pulegone have positive values compared to the controls. Moreover, all the FDI values are between 50% and 70%, which proves that the two essential oils and the pulegone are moderately deterrents [36].

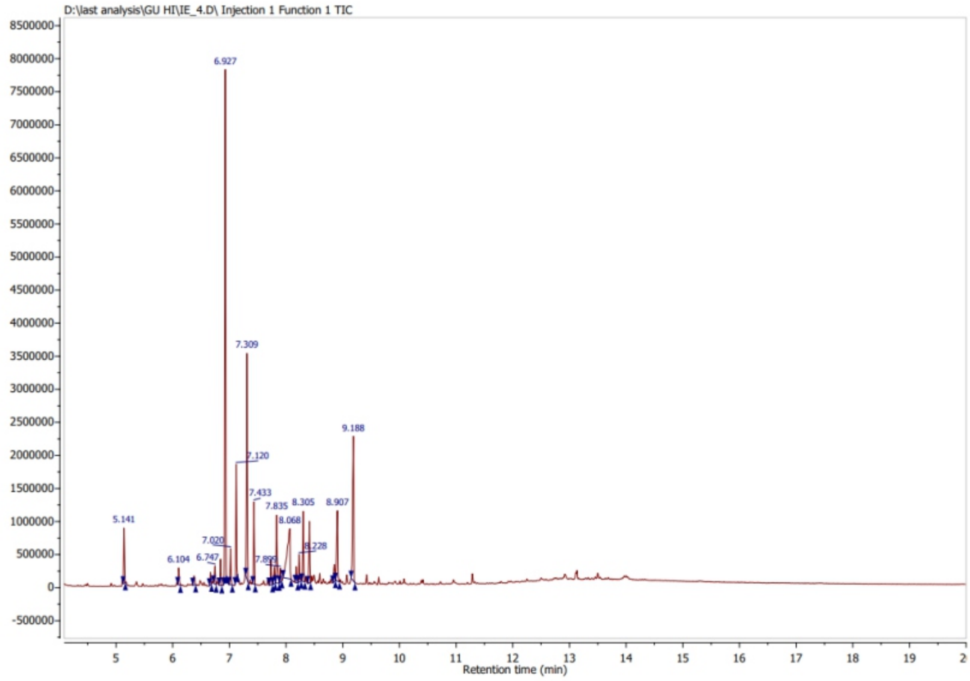

Figure 1. Chromatogram of the Ziziphora hispanica essential oil.

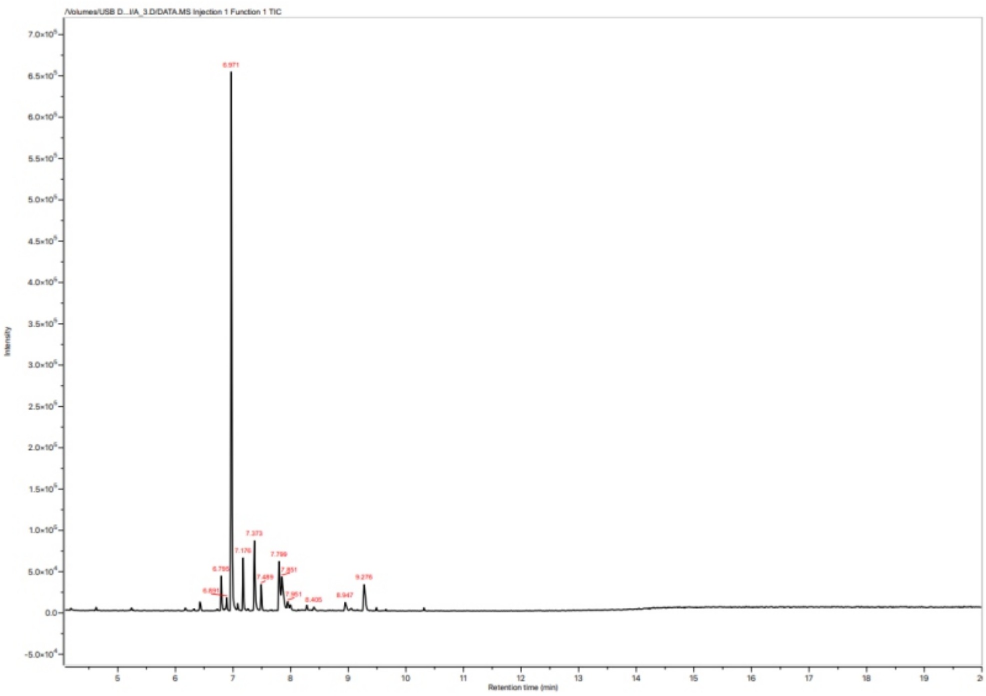

Figure 2. Chromatogram of the Mentha pulegium essential oil.

Table 1. Chemical composition of EOZH and EOMP.

Compounds	RI_{exp}	RI_{th}	EOZH(*)	EOMP(**)
α-Pinene	930	942	0.2	1.1
Oct-1-en-3-ol	958	970	0.1	-
β-Pinene	970	974	0.4	-
Sabinene	977	977	0.1	-
Myrcene	979	987	0.1	1.2
α-Phellandrene	1008	1000	-	1.4
p-Cymene	1010	1007	0.2	-
1,8-Cineole	1019	1016	0.2	-
Limonene	1010	1021	3.5	5.4
Eucalyptol	1021	1016	0.3	0.3
γ-Terpinene	1047	1252	0.2	0.1
p-Mentha-3,8-diene	1058	1060	0.2	-
Isomenthone	1171	1170	-	0.5
Terpinolene	1077	1077	0.1	0.8
Linalool	1081	1085	0.1	0.4
p-Mentha-2,8-diene-1-ol	1101	1124	0.2	0.5
Menthone	1129	1127	-	9.1
Verbenol	1137	1138	0.1	0.2
4-Menthen-8-ol	1140	1140	2.8	-
Pinocarveol	1142	1141	0.6	0.2
Borneol	1145	1143	-	0.7
Menthofurane	1146	1147	0.9	0.1
trans-Isopulegone	1148	1152	0.8	-
p-Menthane-3-ol	1155	1158	0.2	2.1
cis-Isopulegone	1159	1159	1.2	-
Terpineol-4	1160	1162	0.1	0.1
α-Terpineol	1172	1172	0.2	-
Myrtenol	1175	1173	-	0.2
trans-Carveol	1196	1192	0.2	-
Pulegone	1208	1208	79.1	63.8
Piperitone	1223	1221	0.2	0.7
cis-Carveol	1226	1225	0.1	0.3
cis-Piperitone oxide	1255	1257	0.2	-
Carvacrol	1272	1279	1.6	-
Thymol	1282	1288	0.9	-
Mintlactone	1314	1314	0.3	-
Piperitenone	1318	1317	0.6	-
(E)-β-Caryophyllene	1417	1416	-	2.1
Germacrene-D	1471	1473	-	4.1
Caryophyllene oxide	1569	1561	0.2	0.4
		Total	96.2	95.8

(*) 33 compounds in the essential oil of *Ziziphora hispanica* (EOZH); (**) 24 compounds in the essential oil of *Mentha pulegium* (EOMP).

Table 2. Insecticidal activity of the two essential oils and the pulegone

Sample concentration (mg/g of biomass of legume)	*Bruchus lentis*	*Bruchus pisorum*	*Bruchus rufimanus*	F-ratio	p-value
LD_{50} (mg/g)					
EOZH	121.4 ± 4.2	135.5 ± 5.1	133.6 ± 1.6	18.89	< 0.0001*
ZOMP	148.1 ± 5.4	163.4 ± 4.5	137.9 ± 8.5	20.33	< 0.0001*
Pulegone	61.6 ± 5.4	74.4 ± 4.0	63.2 ± 4.9	9.73	< 0.0001*

Different letters in the same row indicate significant differences according to Tukey's test ($p < 0.05$). * Values are significant at $p < 0.05$.

More in detail, the FDI of EOZH was highest against Bruchus pisorum (61) and lowest against Bruchus rufimanus (43). The FDI of EOMP was 63, 62 and 53, respectively against Bruchus pisorum,Bruchus lentis and Bruchus rufinanus. The highest values against all insects were found for pulegone: 67 against Bruchus pisorum, 65 against Bruchus lentis and 63 against Bruchus rufimanus (Figure 3).
The RCR of EOZH was highest in Bruchus pisorum and Bruchus rufimanus whereas was lowest in Bruchus lentis. Also for the EOMP the highest RCR values were found for Bruchus pisorum and Bruchus rufimanus whereas for Bruchus lentis was found a half value compared to the one of Bruchus pisorum. The RCR of pulegone for all insects was lower than EOZH and EOMP (Figure 4).
The control showed the significantly highest values for all the studied species. Data of RGBR are reported in the Figure 5.
All treatments evidenced an effect in reduction of the biomass gain rate of insects. As expected, for all species, the control showed the highest RGBR values. Pulegone was the most effective. EOZH and EOMP showed, by and large, the same effect against Bruchus lentis and Bruchus pisorumwhereas EOMP prevailed against Bruchus rufimanus. The ECIF is described in the Figure 6. EOMP was more effective against Bruchus pisorum and Bruchus rufimanus whereas EOZH showed the highest effect against Bruchus lentis. As for all the nutritional indexes, pulegone was the most effective against the three studied species.

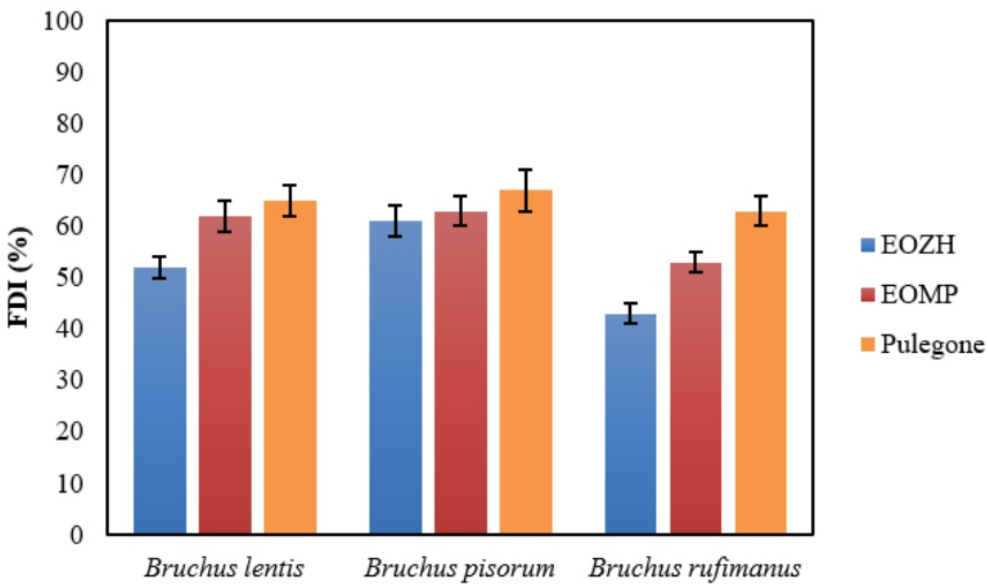

Figure 3. Determination of Feeding-Deterrence Index (FDI).

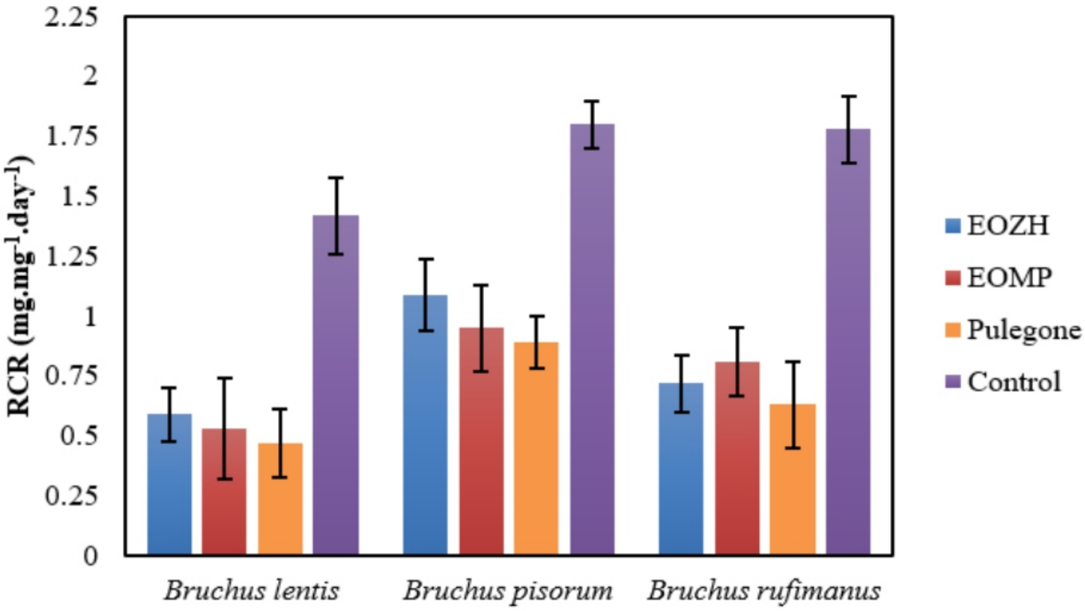

Figure 4. Determination of Relative Consumption Rate (RCR).

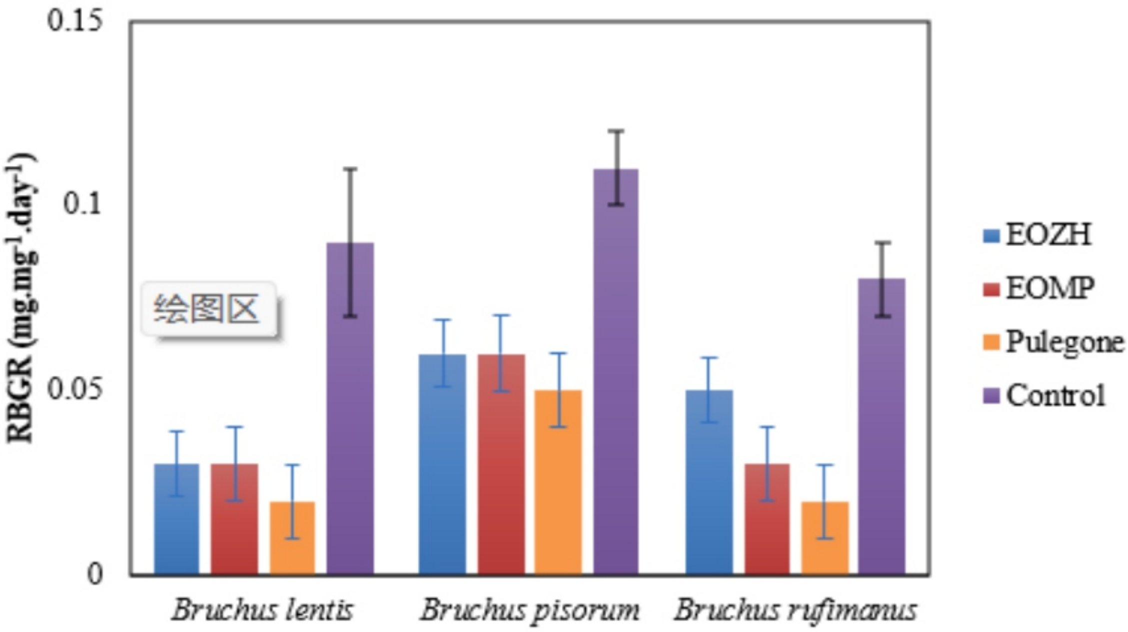

Figure 5. Determination of Relative Biomass Gain Rate (RBGR).

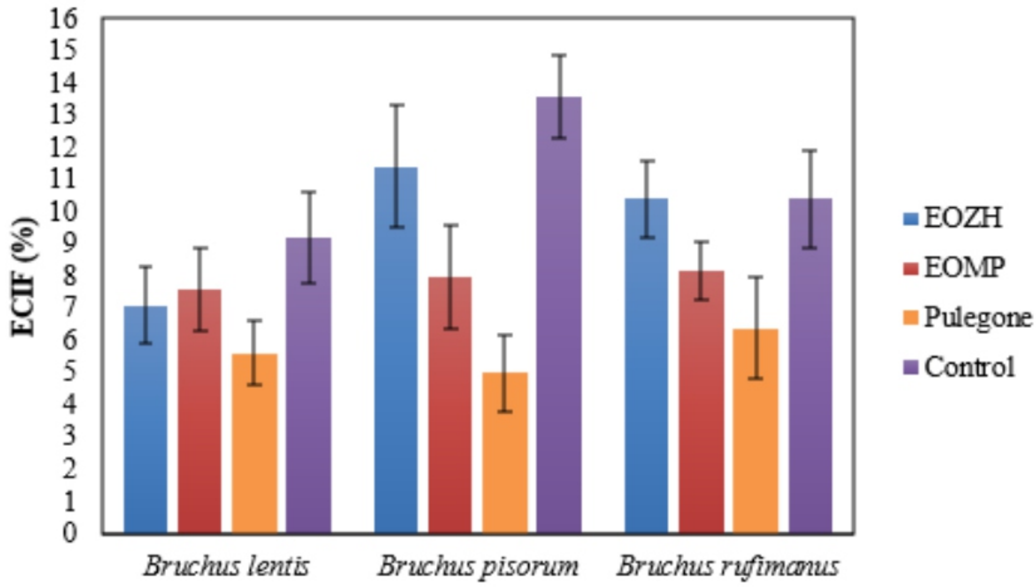

Figure 6. Determination of Efficiency of Conversion of Ingested food (ECIF).

To deepen the study, an additional test was carried out which concerns the biochemical analysis of the essential nutritional values of legumes after a storage period of three months with the essential oils EOZH and EOMP. These analyzes aim to determine the content of proteins, carbohydrates, and total fat, and they were measured according to two modes: without treatment and with treatment with essential oil. All the results are displayed in Table 3.

The statistical analysis by ANOVA of this biochemical contents shows that all the values do not have any significant difference, which proves that the essential oils added do not have a difference on the nutritional quality of stored foods.

Table 3. Biochemical analysis of the essential nutritional values of stored legumes.

Leguminous vs insect	Treatment/statistical analysis	Proteins content	Carbohydrates content	total fat content
Lentil (*Lens culinaris* L.) vs *Bruchus lentis*	Without treatment	9.1 ± 0.1	19.9 ± 0.2	0.41 ± 0.02
	Treatment with EOZH	9.0 ± 0.2	20.1 ± 0.4	0.40 ± 0.01
	After treatment with EOMP	9.0 ± 0.2	20.0 ± 0.4	0.41 ± 0.03
	F-ratio	0.83	0.02	0.04
	p-value	0.458	0. 974	0.959
Pea (*Pisum sativum* L.) vs *Bruchus pisorum*	Without treatment	4.9 ± 0.1	14.4 ± 0.1	0.41 ± 0.03
	Treatement with EOZH	4.9 ± 0.1	14.2 ± 0.2	0.41 ± 0.02
	After treatment with EOMP	4.9 ± 0.1	14.4 ± 0.4	0.39 ± 0.02
	F-ratio	0.28	0.95	0.85
	p-value	0.756	0.411	0.450
Faba bean (*Vicia faba* L.) vs *Bruchus rufimanus*	Without treatment	8.1 ± 0.1	18.3 ± 0.3	0.73 ± 0.03
	Treatment with EOZH	8.0 ± 0.2	18.2 ± 0.3	0.72 ± 0.03
	After treatement with EOMP	8.1 ± 0.1	18.2 ± 0.2	0.73 ± 0.03
	F-ratio	1.31	0.61	0.02
	p-value	0.303	0.612	0.983

Different letters in the same row indicate significant differences according to Tukey's test ($p < 0.05$).

4. Discussion

Natural products remain an inexhaustible source of complex and diverse structures given the role that certain pure compounds can play in many applications, including the food industry. Plants synthesize several substances of secondary metabolism [37,38]. These molecules can have different effects in insects (repellent, attractive, disruptive of development, inhibitor of reproduction, etc.). Their toxicity can be direct or indirect on the target organs [39].

In recent years, and in the face of climate change and restrictive international legislation on the application of synthetic insecticides, the search for natural alternatives is part of a vision adapted tothe requirements of the consumer and the environment [40]. In this strategy, our study directed towards the evaluation of the insecticidal activity of essential oils of Ziziphora hispanica L. and Mentha pulegium L. which are rich in pulegone. The insecticidal effectiveness of these two oils was confirmed by the presence of this molecule, whose activities are important appeared in all bruchids of storage legumes. According to previous works this molecule is reported as a natural insecticide [41–43], so it has presented good effects according to the observed mortalities and high toxicity and is lethal listed in dozens of insect species. The pulegone has specific physico-chemical characters, it is well known that it is not volatile than other organic compounds, but it is highly lipophilic, tending to be more toxic, moreover this type of compounds is less selective in the binding to proteins, being in some cases chemically reactive and extensively metabolized [44,45].

This study concluded that the essential oils of Ziziphora hispanica L. and Mentha pulegium L. well controlled the development of bruchids of leguminous plants with greater attention can be paid to the existence of pulegone as the active ingredient.

The results obtained in this study showed that the use of essential oils as a biological insecticide could be incorporated into the management program for the control of weevil pests as a safe alternative form. Studies conducted in Algeria on the essential oil extracted by hydrodistillation of aerial parts of Ziziphora hispanica revealed pulegone as the major component (78.6%) and menthofurane and limonene accounting respectively for 1.26% and 1.06% of the total essential oil composition [46]. The authors found that essential of the Ziziphora hispanica showed an inhibition activity against Gram positive and Gram negative bacteria and also against fungus and that the inhibition effect was higher in Gram positive bacteria with respect the Gram negative ones [46].

Attia et al. showed an insecticidal effect of M. pulegium essential oil against three scale insects in Le Kef region citrus orchards of Tunisia. They found that hydrodistilled extracts had high toxicity toward Planococcus citri, Aonidiella aurantii and Chrysomphalus aonidum nymphs, whereas they generated low mortality rates of Cryptolaemus montrouzieri adults, a coccinellid predator [47].

5. Conclusions

In conclusion, our data in this study showed that pulegone could act as a potential insecticide, as it exhibited higher activity in insecticidal tests against legume bruchids as well as their specific effects during food storage through the preservation of nutritional biochemical values. Moreover, essential oils rich in this compound revealed a direct relationship with these insecticidal activities. All the products tested were potential tools to control bruchids during storage, but the presence of pulegone improved the bioactivity, which suggests using these natural substances for the control of legumes during the storage period.

Conflict of interest

The authors declare no conflict of interest.

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Causal nexus between agricultural credit rationing and repayment performance: A two-stage Tobit regression

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A B S T R A C T

The probability that the beneficiary would default on future payments poses a great risk to extending agricultural credit. Also, previous research on farmers' repayment of agricultural credit emphasized that a high default rate is a growing concern, thereby becoming a tall order for financial institutions to lend to farmers. Similarly, past studies accentuate an increasing focus on socio-economic characteristics as factors that explain the repayment rate. The nexus between repayment rates and credit rationing has not been well analyzed. The effect of credit rationing on repayment rate was therefore investigated. The study, therefore, investigates the causal effects of credit rationing on loan repayment performance using a structured questionnaire to elicit information from selected 240 respondents via a three-stage method of sampling technique, and the instrumental variable Tobit technique to analyze the effect of credit rationing on repayment performance. The result showed that the majority (70.83%) of the respondents are males, the mean age was 51 years with an average education year of 12.65. The result of instrumental variable Tobit regression confirmed the endogeneity of rationing rate (Wald test of exogeneity = Wald Chi2 (1) = 67.26; Prob > chi2 = 0.000) at a 1% level of statistical significance. The result with a Log-likelihood function (265.62459) revealed that the ration rate, among others, with coefficients of 0.4335, was a crucial factor in ascertaining the rate of repayment at various significant levels of the arable crop farmers in the research area. The key finding is that credit rationing did have a significantly positive influence on agricultural credit repayment. The research concluded that the significance of credit rationing in influencing the likelihood of repayment rate, points to the vital significance of adequacy in rationing borrowers.

Keywords: agricultural credit; credit repayment; credit rationing; endogeneity; Tobit regression;productivity

1. Introduction

In boosting the agricultural development of a country, agricultural credit for farmers plays a major and vital role in promoting productivity [1] in both the short and long terms. It is a global belief that investing in agricultural enterprises through the provision of microcredit will continue to be seen as a potential means for improving the income of farmers. Agricultural or farm credit is defined as a vital input needed by farmers for establishing and expanding their farms with the target of enhancing food adequacy, increasing agricultural production, advancing national and household income, as well as augmenting each borrower's competence to pay back the borrowed funds. Credit is a veritable tool that grants farmers the driving forces to gainfully utilize fixed capital, working capital, and consumption goods [2].

The need for agricultural credit among farmers cannot be exaggerated [3,4], as it enables the establishment and expansion of their farms. The Nigerian Agricultural and Cooperative Bank was incorporated alongside other banks in 1999 to facilitate agricultural production and became an integrated banking structure called the Nigerian Agricultural Cooperative and Rural Development Bank (NACRB). It was intended to accept loans for marketing, distribution, and storage of agricultural commodities associated with such products to a state, collection of states or related institutions for lending to farmers, groups of agricultural practitioners, or business entities subject to the state or collection of states or related institutions assuring loan repayment. In October 2010, the bank took up the new name Bank of Agriculture Limited (BOA), following the rebranding of NACRB to give a reflection on its institutional change programme.

However, with these revived interests in enhancing the status of farmers through credit provision, a crucial issue that has popped up is the subject of credit repayment. Major problems confronting these agricultural loan projects, regardless of the funding channel, are low rates of credit retrieval and patronage. The primary outcome of default in paying back agricultural credit is that it diminishes the viability and vitality of credit or financial organizations. [5] stressed that if the impediments pertaining to the repayment of loans are eradicated, the resolution of the government to foster massive involvement of agricultural practitioners in credit plans is inclined to bear eligible outcomes. There is no agricultural credit available outside a few cost implications. Certain elements are taken into consideration prior to being accessible to the beneficiary, and one of those factors is the beneficiary's aptitude to pay back the borrowed fund, which in turn is having several other determinant factors. According to empirical studies on credit repayment, its performance could be persuaded by a multitude of factors including rate of interest, fluctuating prices of farm produce, and the social affinity and obligations of the borrowers. While several other elements abound, [5] revealed that institutional(lending institutions) factors such as the cost of obtaining a loan, disbursement lag, loan size, and supervision among many others, could significantly assist in boosting the repayment attitude of farmers [6]. The concerns of repayment of a loan that has a collateral bearing on the rate of default should be properly managed to evade misery and a feeble foundation for the establishment of fiscal institutions. One of the impediments to the Nigerian agricultural sector growth that has been carefully noticed is the non-repayment of agricultural credit, as it weakens the readiness of financial institutions to heighten the funding sector.

According to Ugbajah [7], the availability of loans to peasant farmers poses a big problem. This is due to the rate at which defaulting cases among small farmers increase. This is often owned to factors like lack of bankable security, high administrative costs, and the high risks perceived to be connected with agricultural practices and small-scale farmers [8].

1.1. Credit markets and rationing

The credit market varies from the recognized markets for goods and services in two relevant ways. The first variations, as shown by the classical competitive theory, exists based on the fact that in recognized or rather standard markets, buying and selling a homogenous product involves many agents. The second dissimilarity dwells on the fact that the handing over of goods or services and their payment occur concurrently in such markets. Contrastingly, credit acquired currently by one person is used in exchange for an assurance of future repayment. Stiglitz (as cited in Abafita, [9]) opined that since promises vary individually, and are oftentimes broken, there is possibly no actual proof of ascertaining that an agreement will not be violated, implying that, moral hazard and contrary selection could determine the probability that the promise is upheld and hence that of loan repayment.

Since lenders cannot directly influence all decisions made by their borrowers, the condition of the loan contract is formulated in a manner that influences the beneficiary to behave for the benefit of the lender [5]. Due to this, the gain the lender looks forward to may move up less speedily than the interest rate, and farther than a level may assume declination. The demand for credit transcends the loan supply at such a rate of interest when the anticipated profit to the lender begins to decline. The lender would not give out a loan to a person who proposes a higher interest rate because its anticipated return is lesser. Therefore, there is an absence of competitive forces to equate supply with demand; and credit is rationed.

Credit rationing is widely explained as a condition where the loan demand exceeds the loan supply at the existing interest rate. In the literature, various forms of credit rationing were examined by [2] viewed via the viewpoint of the size of loan in which borrowers access a smaller loan amount than requested at a known loan rate. Also, 4 types of credit rationing: Quantity, Transaction cost, Price, and Risk rationing as mentioned in Figure 1 of Anh et al. [10]. Jaffe and Stiglitz (as cited in Abafita, [9]) further widened the classification by identifying three types of credit rationing. Firstly, it is a condition where a borrower obtains a loan of a lesser amount than wanted or requested. Secondly, it is a situation where a borrower is unable to borrow at the interest rate considered inappropriate based on the perception of their default likelihood. Lastly, it is a condition in which a borrower is denied credit when a lender perceives being unable to achieve the expected return regardless of the interest rate. The concept that this research considered is the type one of credit rationing.

1.2. Justification

Accordingly, many loan projects of agricultural credit organizations have broken down because of vast loan repayment arrears [11]. A low rate of credit recovery, such as is observed in many agricultural credit schemes, does not augur well for agricultural financing and likewise for lending institutions. Poor agricultural credit recovery rate generates caution in lending to the sector, and this has adversely affected the on-lending programme of several credit institutions. In Nigeria, the acclaimed importance of agricultural credit in improving the sector as well as affecting the economy positively has more than enough challenges. [12] affirmed that the acquisition, management, and repayment of agricultural credit in Nigeria have been burdened with numerous challenges.

There is no uncertainty that in contemporary times, significant interest has been shown by researchers to look into the problems of agricultural credit repayment among farmers as well as its determinants. However, previous research efforts emphasized certain institutional characteristics such as collateral, cost of credit or rather rate of interest, and disbursement lag, among other several factors. Studies on the influence of loan rationing, as part of institutional characteristics on credit repayment, are scanty. Even rarer in previous work is the attempt to address the endogeneity problem of loan rationing when estimating factors determining credit repayment. For instance, Firafis, [13] examined credit rationing and repayment performance using a binary logit model. However, this study employed the Instrumental Variables (IV) regression method in examining the effect of credit rationing on repayment performance. This approach is more suitable due to its ability to control for the endogeneity of credit rationing in the model, thus eliminating the biasedness of the Ordinary Least Squares (OLS) method.

Viewing from this backdrop, it is perceived that it is a necessity to examine the effect ofagricultural credit rationing on repayment performance while controlling for the endogeneity of credit rationing using the two-stage Tobit regression approach.

1.3. Theoretical background

Extant studies have considered both the impact of agricultural credit on agricultural productivity [14] and how repayment rate can be affected by degree of loan rationing, group size, size of loans, anddisbursement lag, among others [4,11,15–19] examined the impact of socio-demographic and loan related variables using Tobit regression and confirmed the significant effect of land size, experience, income and contact with extension agents on loan repayment rate. [20] have illustrated that "education level, marital status, nationality, employment status, and business activity sector have a significant

impact on borrowers' repayment performance". [21] asserted that the loan amount, purpose of the loan, marital status, education, and monthly income have effect which is significant on the probability of increasing the welfare of borrowers once they receive microcredit. [22] in their work, touched on the fact that borrowers' loan size is being impacted by the cost of microfinance intermediation and recommended that to reduce cost, big loans should be extended to clients having a longer loan experience, high income, lower informal borrowings, assets, and land size.
More importantly is the work of [23] that investigated the effect of some variables such as education, loan size, and training and/or a number of visits on repayment, and concluded a positive significance of education, employment, and high potential cash flow streams on repayment rate. In another related study, [24] asserted that training/professional experience has some positive impact. In a similar study, [25], indicated that a higher income decreases the probability of default in repayment. [26] observed that socio-demographic and loan-related factors can determine whether a borrower would repay a microcredit. It specifically indicated the positive significance of age, experience, amount of credit, and education on repayment performance.

2. Materials and methods

2.1. Area of Research

The research was conducted in Osun State, south-western Nigeria. The State has thirty (30) Local Government Areas (LGAs) and is parted into three agricultural zones, namely: Iwo agricultural zone, Osogbo agricultural zone, and Ife/Ijesha agricultural zone. The LGAs covered in this study are enriched with agro-climatic and soil-type factors suitable for cultivating arable crops such as cassava, maize, yam, potato, and diver vegetables.

2.2. Source and type of data

The research utilized primary data gathered using a well-designed questionnaire that captured both socio-economic and farm features of arable crop farmers. These consist of age, household size, sex, years of education, farming experience, farm size, and some other relevant information on agricultural credit.

2.3. The population of the study

The population for this study was farmers' cooperative groups who obtained farm credit support from the Quick Impact Intervention Programme (QIIP), out of which farmers who cultivate arable crops were selected. The farmers' cooperative groups that cultivated arable crops and benefitted from QIIP credit support were 205 cooperative groups, 50 groups were selected for the study.

2.4. Sampling procedure and sample size

A three-stage sampling technique was used for this research. Firstly, five Local Government Areas (LGAs) noted for the highest number of arable crop farmers' and the cooperative groups who participated in the credit support scheme as recorded by QIIP were purposively selected. The selected LGAs are: Osogbo (47), Iwo (30), Ede North (27), Egbedore (23), and Ayedaade (20). Secondly, farmers' cooperative groups were randomly selected based on the proportion of the cooperative groups in each local government area. Using a proportionate-to-size sampling, 16 groups were randomly selected in Osogbo LGA, 10 groups in Iwo LGA, 9 groups in Ede-North LGA, 8 groups in Egbedore LGA, and 7 groups in Ayedaade LGA. Finally, 5 respondents were randomly sampled in each of the selected groups in the 5 LGAs to arrive at a total of 250 respondents.

It should, however, be noted that only two hundred and forty (240) copies of the questionnaire were used in the analyses. Inadequate information and inconsistency necessitated the rejection of others.

2.5. Instrumental variable Tobit regression analysis

Agricultural credit repayment or rather repayment performance of farmers has been diversely studied and reported in the literature, but there is very little evidence of a possible endogeneity of loan rationing and its influence on repayment performance. The problem of endogeneity encountered in studying the influence of loan rationing on agricultural credit repayment performance is outlined. Thus, following [27], and Ben [28], the recommended approach to deal with the problem of endogeneity in any econometric model is through instrumental variable techniques. Instrumental variable Tobit regression (ivtobit) fits models with censored dependent variables as well as endogenous covariates. It is used to fit a Tobit model when it is suspected that there is a correlation between the error term and one or more of the covariates.

Formally, the model is:

$$Y^*_{1i} = Y_{2i}\beta + X_{1i}\Upsilon + u_i \qquad (1)$$

$$Y_{2i} = X_{1i}\Pi_1 + X_{2i}\Pi_2 + v_i \qquad (2)$$

Where: i = 1, …, N; Y2i = 1 × p vector of endogenous variables; X1i = 1 × k1 vector of exogenous variables; X2i = 1 × k2 vector of additional instruments; β and ϒ = vectors of structural parameters; and Π1 and Π2 = matrices of reduced-form parameters; Y*1i is unobserved.The log likelihood is of the form:

$$\ln L_i = w_i\{\ln f(Y_{1i}|Y_{2i}, X_i) + \ln f(Y_{2i}|X_i)\} \qquad (3)$$

Where wi is for observation “in” or one if no weight specifications were made.

2.5.1. Model specification

In analyzing the effect of credit rationing on repayment performance, the specification for the instrumental variable tobit model is specified as :

$$RPYMNT(Y) = \beta_0 + \beta_1 SEX + \beta_2 EDUC + \beta_3 HHS + \beta_4 FARMEXP + \beta_5 AMTGRANTED + \beta_6 COC + \beta_7 DBMTLAG + \beta_8 NFI + \beta_9 VST + \beta_{10} RATION + \ell \qquad (4)$$

Where: RPYMNT(Y) = Repayment rate (proportion of loan repaid as at when due) explained asAmount repaid/Total amount of loans obtained; SEX = Sex of the beneficiary (male = 1, and female= 0); EDUC = Education (years); HHS = Household size (Numbers); FRMEXP = Farming experience (years); AMTGRANTED = Amount granted (Naira); COC = Cost of credit (percent); DBMTLAG = Disbursement lag (days); NFI = Net farm income (Naira); VST = Number of visits by QIIP officials (number); RATION = Rationing rate; ℓ = Error term.

However, it is supposed that the ration rate is endogenous to the repayment rate, and failure to control for the problem of endogeneity leads to biased parameter estimates. An instrumental variables approach was therefore used to account for the possibility that the rationing rate is endogenous to repayment performance. The study assessed the validity of the instruments and then used age, farm size, previous farm income, saving habits, and extension services as the instruments for rationing rates. Based on this thought, the empirical model is given as:

$$RATION(Y) = \beta_0 + \beta_1 AGE + \beta_2 FRMSIZE + \beta_3 PREVINC + \beta_4 SAV + \beta_5 EXT + \ell \qquad (5)$$

Where: RATION(Y) = Ration rate (proportion of loan sized ration) defined as 1 – (Amount granted/Amount requested) or 0 otherwise; AGE = Age of the farmer (years); FRMSIZE = Farm size (hectares); PREVINC = Previous Income of the farmers in the previous season (Naira); SAV = Saving habit (Yes = 1, otherwise = 0); EXT = Extension (Yes = 1, otherwise = 0); ℓ = Error term.

3. Results and discussion

3.1. Socio-economic characteristics

As shown by the results in Table 1, male farmers accounted for 70.83 percent, while the remaining 29.17% were females, implying that men engaged more in farming practices than women in the study area.

The influence of age in conventional agriculture is relevant in two suppositions. The first is higher productivity, whereas the second is concerned with a better adoption level of innovations. Aged farmers are probably to be more conservative and fortify themselves to the unresistant influence of change engaging young farmers. The average age was 51.05 years which indicates that farmers are beyond their economic active or productive age. The result corresponds to the findings of Afolabi [29] who had earlier reported that age groups above 50 years are beyond their economic active or productive age.

The importance of education in agriculture cannot be overemphasized, since the years of education of a farmer are not only important for increased productivity but also positively affect the adoption of new agricultural techniques and likewise enhances the ability to understand and evaluate new production techniques. The level of literacy among the sampled farmers contained in the study is high with an average education year of 12.65 as shown in Table 1.

Table 1. Socio-economic characteristics of the beneficiaries.

Characteristics	Frequency	Percentage (%)
Age (Years)		
<40	29	12.08
40–50	86	35.83
51–60	89	37.08
>60	36	15
Total	240	100
Average age = 51.05		
Std. Dev = 9.14		
Sex		
Female	70	29.17
Male	170	70.83
Total	240	100
Education Years		
6	32	13.33
7–12	72	30
13–17	129	53.75
>17	7	2.92
Total	100	100
Average = 12.65		
Std. Dev = 3.57		
Household size		

3–5	113	47.08
6–8	127	52.92
Total	100	100
Std. Dev = 1.22		
Average = 6		

Source: Field Survey, 2016.

From the results in Table 1, the majority (52.92%) of the beneficiaries have a household size of six to eight. This implies that a higher percentage of the respondents have household sizes that have the chance of raising their total expenses. On the other hand, bigger households could function as a labour source which could increase the output of the farmers, reduce cost, and hence increase the income level. However, engagement in farm work depends on the age structure of household members. The average household size stood at approximately 6 persons per household during the study. [17] accounted for an average farming household size of 7 persons in their study area.
The number of years spent in farming practices could indicate the knowledge and technical ideas on how to tackle farm production problems. Farmers with more years of farming experience may achieve efficiency via trial and error. As revealed in Table 2, the sampled beneficiaries have gained the necessary experience needed in arable crop production with an average year of 22.35.

3.2. Instrumental variable Tobit Regression results in credit rationing on repayment performance

Table 3 presents the second-stage results of the instrumented variable Tobit model. A total of 10 exogenous variables were considered in the econometric model, of which 7 were statistically significant at various levels. The significant variables are ration rate, farm experience, household size, the amount granted, disbursement lag, cost of credit, and net farm income. The variables used as instruments for ration rate were age, farm size, previous farm income, extension, service access, and saving habits. The Wald chi-square of 139.69 with a p-value of 0.0000 reveals the model fitness, and it has a log-likelihood of 265.62459.
The result of the Wald test of exogeneity of the instrumented variable with chi2 (1) = 67.26 and Prob > chi2 = 0.0000 was statistically significant and at 1% level of significance. This goes on to suggest that the null hypothesis that the rationing rate is exogenous in the repayment performance equation is rejected at a 1% significant level. Thus, justifies the use of instrumental variable Tobit (ivtobit) regression.

Table 2. Descriptive for years of farming experience and rationed borrowers.

Characteristics	Frequency	Percentage (%)
Farm experience years		
<5	5	2.08
5–15	70	29.17
16–25	77	32.08
26–35	53	22.08
>35	35	14.58
Average = 22.35		
Std. Dev = 10.72		
Total	240	100
Loan Ration		
Non-rationed beneficiaries	62	25.83
Rationed beneficiaries	178	74.17
Total	240	100

Source: Field survey, 2016.

The coefficient for ration rate was positive and at 1% level of statistical significance. The result implies that increasing the rationing rate by one percent raises the likelihood of higher repayment performance by 0.4335 among the sampled crop farmers in the research area. This suggests rationing agricultural credit beneficiaries effectively and extending adequate loan size as per their credit needs enables the farmers to put the credit to effective use, curbs misappropriating credit, and hence ups the repayment performance of the sampled beneficiaries. The result agrees with the discovery of Firafis [13] who found that credit rationing positively influences loan repayment performance.

Household size has a negative effect on the repayment performance of the arable crop farmers in the study area and is statistically significant at the 5% level of significance. Increasing the size of farmers' households by one person decreased the likelihood of credit being repaid by 0. 0163. The household size coefficient conforms with a priori expectation in that a larger household size probably increased the financial obligations of the sampled farmers in the research area. Hence, the responsibility imposed by a larger household size was probable to compress the agricultural resources from which loan repayment could be made. The result corroborates with the findings of Afolabi [29]and [5,30] who in their separate studies discovered that household size has a negative impact on farmers' repayment performance in their respective study areas.

Moreover, the variable years of farming experience of the sampled arable crop farmers conform to the a priori expectation that the more experienced farmers have played a vital role in enhancing their farming activities and practices, which can raise their income level and hence credit repayment performance. The result in Table 3 shows that farming experience influences the repayment rate positively at the 5% level of statistical significance. This depicts that increasing years of farming experience by year raises the likelihood of credit repayment by 0.0025 among the sampled arable crop farmers in the research area. This confirmed the findings of Afolabi [29] in his study "analysis of loan repayment among small-scale farmers in Oyo State, Nigeria".

Amount granted significantly influenced the repayment rate positively at 1% level of statistical significance. An increase in the amount granted by one Nigeria naira increased the likelihood of repayment rate by 1.61e−06 among the sampled arable crop farmers in the research area. This connotes that the bigger the loan size, the more money the farmers have to invest in farming activities, which could as well lead to higher chances of adopting advanced technology which could heighten the income capacity generation of the farmers, and hence lead to more loans being repaid. A similar positive influence of the amount granted on repayment performance was reported by Ojiako and Ogbukwa [30].

The cost of credit significantly influenced the repayment rate negatively at the 1% level of statistical significance. As shown by the results in Table 3, an increase in the cost of credit by one unit decreased the likelihood of repayment rate by −1.18e−09 among the sampled respondents in the study area. The negative sign conforms to a priori expectation in that the higher cost of the loan will reduce the repayment rate of borrowers. The result corroborates those of [31]; and [30]; who in their separate studies found that the cost of loans impacted negatively farmers' loan repayment performance in their study area.

The variable disbursement lag had a negative effect on the repayment rate at 1% percent level of statistical significance. The estimates in Table 3 show that an increase in the cost of credit reduced the likelihood of repayment rate by 0.0045 among the sampled arable crop farmers in the study area. This goes on to buttress the fact that farm operations, as well as agricultural production, are time-bound, and if loans meant for agricultural production are not disbursed timely and delayed beyond the critical production period, such will be irrelevant for production and underutilized, thus stimulating a low repayment rate. The result is in agreement with the findings of Oke et al., [32] who in their study discovered that disbursement lag impacted the repayment rate negatively.

Meeting a priori net farm income influenced the repayment rate positively at 5% level of statistical significance. An increase in net farm income by one Nigeria naira increased the likelihood of higher repayment rates by 1.29e−07 among the sampled farmers in the study area. This implies that farmers who earn better income from their farm products give more consideration to loan repayment. The results buttress the findings of [33] who in their study found that the net farm income of farmers impacted the repayment performance positively.

Table 3. Second stage result of instrumental variable Tobit parameter estimates of the effects of rationing on repayment rate.

Variables	Coefficient	Robust std. error	z-stat	P > |z|
Rationing rate	0.4335***	0.0444	9.76	0.000
Sex	0.0183	0.0234	0.78	0.434
Education years	0.0034	0.0026	1.33	0.184
Household size	−0.0163*	0.0085	−1.92	0.055
Farm experience	0.0025**	0.0012	2.01	0.044
Amount granted	1.61e−06***	2.84e−07	5.65	0.000
Cost of credit	−1.18e−09***	3.41e−10	−3.47	0.001
Disbursement lag	−0.0045***	0.0008	−5.81	0.000
Net farm income	1.29e−07**	5.66e−08	2.27	0.023
Supervision	0.0073	0.0167	0.44	0.663
Constant	0.2817	0.0846	3.33	0.001

Source: Field survey, 2016. ***, ** and * significant at 0.01, 0.05, and 0.1 levels, respectively. Wald chi2 (9) = 139.69; Prob > chi2 = 0.0000; Log likelihood = 265.62459; Wald test of exogeneity of instrumented variables (corr = 0): chi2 (1) = 67.26; Prob > chi2= 0.0000. Instrumented: Rationing rate.

The elasticity decomposition of the value expected for credit repayment for QIIP in the area of study is revealed in Table 4, The estimated elasticities from the model revealed that the marginal alteration in diver characteristics raises the expected value of credit repaid more than it increases the probability for credit repayment.

Table 4. Elasticity of repayment rate.

Variables	Probability of loan repayment	Expected value of repayment rate	Total Elasticity
Rationing rate	0.0004	0.0050**	0.0054
Sex	0.0000	0.0001	0.0001
Education years	7.37e−06	0.0001	7.3705
Household size	−0.0001	−0.0009***	−0.001
Farm experience	0.0000	0.0001**	0.0001
Amount granted	2.83e−09	3.35e−08**	6.1817
Cost of credit	−3.22e−12	−3.81e−11	−7.0323
Disbursement lag	−0.0000	−0.0002***	−0.0002
Net farm income	5.58e−10	6.60e−09	12.1819
Supervision	0.0000	0.0003	0.0003

Source: Field survey, 2016. ***, ** and * significant at 0.01, 0.05, and 0.1 levels, respectively.

4. Conclusions

Justifying the notion that credit rationing rate should not be an exogenous variable in the loan repayment performance model but rather should be viewed as endogenous; using the instrumental variable to bit regression the Wald exogeneity of instrumented variables turned out to be significant at 1% level of statistical significance.

Contrary to the a priori expectation and widely held beliefs, the results showed that credit rationing did have a significantly positive influence on agricultural credit repayment rate, although the sex of the beneficiaries, education years, and the number of visitations from the lending agency did not have significant influence. Years of farming experience, the amount granted, and net farm income positively impacts repayment performance at different levels of significance; while variables like household size, disbursement lag, and cost of credit tend to reduce the likelihood of credit repayment. Traditional variables like sex and education years were not significant in the repayment performance model and hence should not be used as a determinant for credit size. The current research, using fitting model specifications under the assumption that every estimated parameter would stay constant over time, reveals that the type of estimated model will greatly impart information for the assessment of both prospective lending institutions and farmers for loan advantage.

Decomposition of credit repayment elasticity showed that the elasticity of loan value repaid as when due was higher than the elasticity of the probability of loan repayment since the amount of credit size recouped can make significant progress in beseeching the lending competencies of the institutions.

5. Recommendations

The fact that the research confirmed the significance of credit rationing in increasing the likelihood of credit repayment signals the vital importance of adequacy in rationing borrowers, i.e., not, demanding more than what is needed and not giving less than the needed credit size. When credit rationing is done to perfection, beneficiaries will receive a sufficient amount as regards their credit needs and potency to utilize the credit judiciously and therefore are reckoned upon to have high repayment performance. However, it should be noted that, when credit rationing is done for imperfection, borrowers may receive credit amounts that are contrary to their credit needs and their ability to utilize credit, thus resulting in low credit being repaid.

The fact that the study confirmed disbursement lag in reducing loan recovery and loan repayment reflects the effective significance of timeliness in loan negotiation and disbursement. There is a tendency for loan diversion into activities that are relatively less productive or totally unproductive when loan disbursement fails to meet up with farmers' critical time of use. The barriers of inadequately skilled staff, bureaucratic protocols, and rigid requirements for fulfillment before disbursement and disbursement in installment, which is often causes of delay, must be eliminated to permit an effective functioning of the credit market. Hence, lending institutions should imbibe timely discharge of funds, acknowledging the fact that farming activities are extremely time specific

Conflict of interest

All authors declare no conflicts of interest in this paper.

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The comparative analysis of agronomic, compositional, and physiological traits of miraculin transgenic tomato in the confined field trial

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ABSTRACT

The miraculin transgenic tomato is a genetically modified (GM) crop that can be used as an alternative for low calories food and a natural non-sugar sweetener. Before the release and distribution, transgenic crop needs to go through an environmental risk assessment (ERA) as a backbone to achieve biosafety. Comparative analysis is a general principle of ERA to identify differences between transgenic crop and its non-transgenic counterpart which may indicate substantial equivalence and unintended effects. This experiment was aimed to compare the agronomic, compositional, and physiological characteristics of miraculin transgenic tomato cv. Moneymaker with non-transgenic tomato. The data obtained were plant height, stem diameter, relative growth rate, chlorophyll content, stomatal conductance, days to 50% flowering, days to fruit maturity, a number of flowers per cluster, a number of fruits per cluster, a number of fruits per plant, fruit weight, fruit diameter, harvest index, total dissolved solids, fructose, glucose, and sucrose contents, and total carotenoids, lycopene, and β-carotene contents. This study found that there were no significant differences between miraculin transgenic and non-transgenic tomato in all variables observed. It suggests that miraculin transgenic tomato is equivalent to its counterpart and unintended effects are not detected as.

Keywords: environmental risk assessment; limits of concern; substantial equivalence; tomato; unintended effects

1. Introduction

The miraculin transgenic tomato is a genetically modified (GM) crop that expresses the miraculin gene. This gene was isolated from miracle fruit (Synsepalum dulcificum) and transferred into the genome of the tomato plants cv. Moneymaker mediated by Agrobacterium tumifaciens vector [1,2]. Miraculin is a glycoprotein compound that is able to turn sour into sweet taste by binding to taste receptors on the tongue [3,4]. Miraculin transgenic tomato can be used as an alternative food that is low in calories and natural non-sugar sweeteners, especially for diabetics [5].

The release, distribution, and utilization of transgenic crops are determined by regulatory permits since transgenic crops would have effects on the environment, human health and animal health [6]. In Indonesia, Government Regulation No. 21 of 2005 lays out that environmental risk assessment (ERA) is required to prevent the occurrence of adverse risks to biodiversity which may due to the use of transgenic crops. This potential risk is associated with unintended effects, the alteration of plant agronomic traits [7] such as dwarfism, delayed flowering, and decreased productivity [8–10].

Composition and physiological characteristics can also change which is caused by the alteration in synthesis of certain proteins as a result of transgene insertion [11,12]. The potential risk can be assessed by comparing the agronomic, compositional, and physiological characteristics of transgenic plants with its conventional counterparts. These characteristics have to be equivalent except for the modified traits [13].

Minister of Environment of the Republic of Indonesia has issued Regulation Number 25 of 2012 regarding ERA which states that ERA is a stepwise process beginning with testing in laboratory, biosafety containment, to confined field trials (CFT). This regulation is in line with the European Food Safety Authority (EFSA) principle. Field testing of transgenic crops is needed to evaluate the expression of target genes and phenotypic characteristics of plants in actual conditions [13].

Previously, Carsono et al. [14] have evaluated the agronomic characteristics of miraculin transgenic tomato cv. Moneymaker and its origin tomato in biosafety containment which show substantial equivalence between transgenic and non-transgenic tomato plants. Until now there is no report regarding on biosafety assessment of miraculin transgenic tomato on agronomic, compositional and physiological traits that conducted in the confined field trial. As one of the important steps in environmental risk assessment, the further evaluation of transgenic crops in CFT is required to be conducted. The objective of this research was to compare the agronomic, compositional, and physiological traits between miraculin transgenic tomato and non-transgenic tomato in CFT. This study will provide substantial equivalence information and possible unintended effects for further utilization and production of miraculin transgenic tomato.

2. Materials and methods

This experiment was conducted in the CFT at Ciparanje experimental station, Jatinangor, West Java Province, Indonesia, during August-December 2020. The CFT was 780 m above sea level and received 156.63 mm of rainfall monthly, with a daily average temperature of 12.3 ℃ minimum and 32 ℃ maximum and mean relative humidity of 84%. The soil type was inceptisols with neutral pH (6.8), medium C-organic content (2.14%), and medium total N, K2O, and P2O2 (0.21%, 31.47 mg/100g−1,13.97 ppm P). The isolation distance was more than 20 m, in accordance with theimplementation regulation on the safety assessment of GMOs in Indonesia. The experiment was arranged in a randomized block design (RBD) with two treatments: miraculin transgenic and non transgenic tomato cv. Moneymaker. Each treatment was replicated 16 times. Experimental plot was 1.2 m long and 6 m wide with a spacing of 60 cm x 60 cm. An experimental unit consisted of 20 plants with a total of 640 plants.

Before sowing, the tomato seeds were soaked in warm water and 70WP propineb fungicide solution for 12 hours and 30 minutes. The seeds were sown in portray with the mixture of soil and cow manure in a 2:1 ratio. After 6 weeks, the seedlings were transferred into the field. Fertilization using NPK 16:16:16 was applied at transplanting, 30, and 60 days after planting (DAP). Plants were watered once in two days. Manual weeding was done 3 times during the planting season. The stakes were installed when the tomato plants were 21 DAP or 3 weeks after planting (WAP). Tomato was harvested 3 times at the breaker stage phase with intervals of 5 days.

The agronomic traits observed were plant height, stem diameter, relative growth rate (RGR), days to 50% flowering, days to fruit maturity, a number of flowers per cluster, a number of fruits per cluster, a number of fruits per plant, fruit weight per item, fruit diameter, and harvest index. The compositional analysis consists of total dissolved solids (TDS), fructose, glucose, and sucrose contents, and total carotenoids, lycopene, and β-carotene contents. TDS was determined using refractometer (Atago Model 41325). Sugar contents was analyzed by High Performance Liquid Chromatograph (HPLC) method and total carotenoids, lycopene, and β-carotene contents were analyzed using spectrophotometric method. The physiological traits consist of leaf chlorophyll content and stomatal conductance. Chlorophyll content was measured with chlorophyll meter (CCM-200 Plus). Leaf stomatal conductance was measured using leaf porometer (Decagon device, Inc.). The data were analyzed using the independent samples t-test and Limit of Concern (LoC) [6] as presented below.

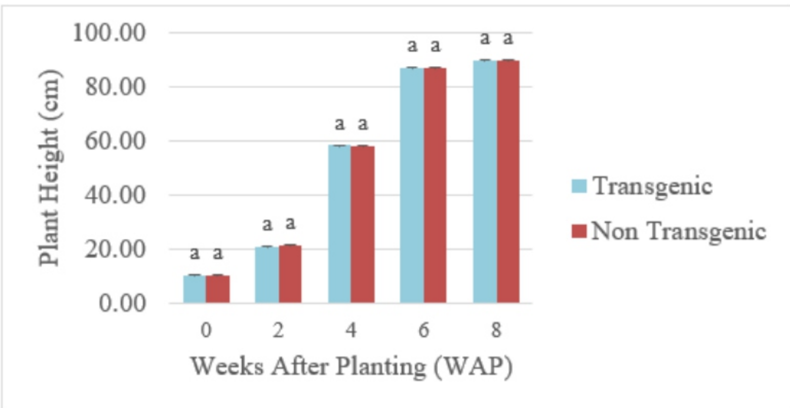
$$0.5 \leq \frac{\text{Transgenic Plants}}{\textit{Non Transgenic Plants}} \leq 1.5 \qquad (1)$$

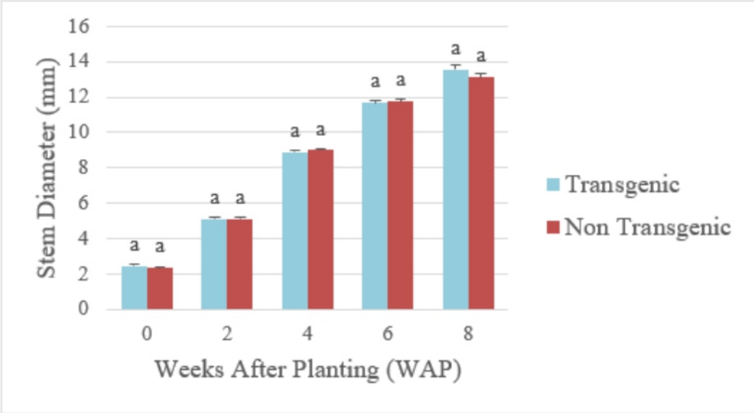
Value > 1.5 = Not equivalent (there is an unintended effect);
Value 0.5–1.5 = Equivalent (there is no unintended effect);
Value < 0.5 = Not equivalent (there is an unintended effect).

3. Results and discussion

EFSA suggests using comparative assessment as a starting point for GMOs' whole risk assessment process. The characteristics of the GM plant are compared with those of its conventional counterpart cultivated under similar conditions. The comparative approach's underlying assumption is that traditionally cultivated non-GM plants have a history of being safe for humans, animals, and the environment [13]. This study was a step to ensure that the miraculin transgenic tomato is as safe as its counterpart. Through risk communication, this kind of information was important to generate consumer acceptance of transgenic food [15].

The results demonstrated that there were no significant differences between miraculin transgenic tomato and non-transgenic tomato in height, stem diameter, and relative growth rate variables (Figure 1). No statistical differences were found in a number of flowers and a number of fruits per cluster (Table 1),days to 50% flowering, and days to maturity. The traits of a number of fruits per plant, fruit weight, fruit diameter, and harvest index did not show 'any significant differences (Table 2). The days to 50% flowering of miraculin transgenic and non-transgenic tomato revealed similar results in which the two crops flowered on average 28 DAP. There were also similarities in days to maturity between the two crops at 70 DAP. The mean value of fruit set for both crops was 70.97% and 68.51%, respectively indicating that 29.04%–31.49% of the flowers failed to become fruit. The reduced fruit set could be affected by high temperatures and humidity that cause lower pollen viability and release [16,17].





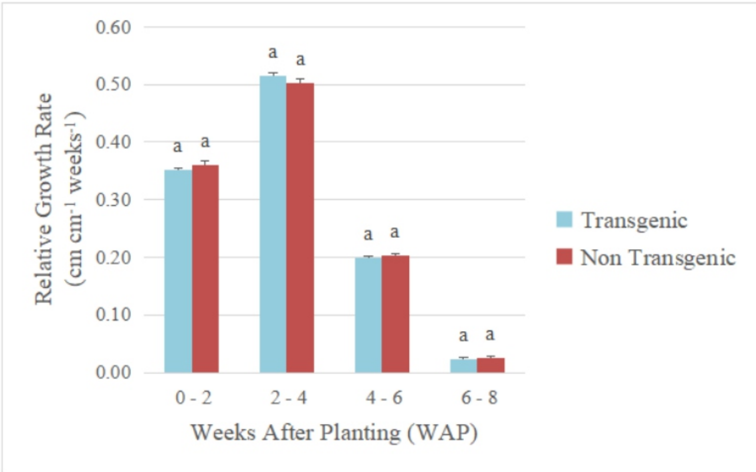


Figure 1. Plant height, stem diameter, and relative growth rates. Values with the same letter in the same column are not significantly different according to student's t-test. Data show mean values with standard error of the means (n = 80)

Table 1. Number of flower and fruit per cluster of transgenic miraculin tomato plants and non-transgenic miraculin tomato plants cv. moneymaker.

Plants	Number of flowers per cluster	Number of fruits per cluster
Transgenic	4.65 ± 0.11 a	3.30 ± 0.08 a
Non-Transgenic	4.51 ± 0.10 a	3.09 ± 0.08 a

Values with the same letter in the same column are not significantly different according to student's t-test. Data show mean values with standard error of the means (n = 80).

Table 2. Total fruit, fruit weight, fruit diameter, and harvest index of transgenic miraculin tomato plants and non-transgenic miraculin tomato plants cultivar moneymaker.

Plants	Number of fruits per plant(fruit)	Fruit weight (g)	Fruit diameter(cm)	Harvest index
Transgenic	14.32 ± 0.26 a	31.79 ± 0.45 a	30.99 ± 0.29 a	0.30 ± 0.01 a
Non-Transgenic	13.90 ± 0.27 a	32.94 ± 0.40 a	31.81 ± 0.34 a	0.27 ± 0.01 a

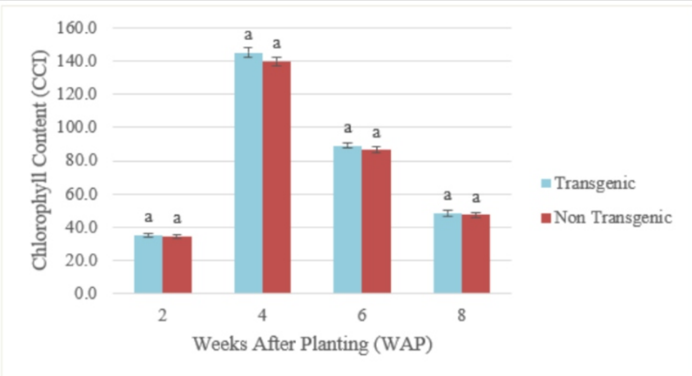
Values with the same letter in the same column are not significantly different according to student's t-test. Data show mean values with standard error of the means (n = 80).

The statistical test showed that there were no significant differences in total dissolved solids, sugar contents, total carotenoids, lycopene content, and β-Carotene between the miraculin transgenic and non-transgenic tomato (Table 3). Chlorophyll content and stomatal conductance results were also not statistically different (Figure 2). This may be because of transgene does not affect biosynthesis pathway of the compound. The research conducted by Kusano et al. (2011) showed that the detected metabolites of miraculin transgenic tomato has 86% of chemical diversity listed in Solanum lycopersicum (LycoCyc) database, which indicates the equivalence of transgenic lines with its control [18]. Based on the limit of concern, the agronomic, compositional, and physiological traits of miraculin transgenic tomato and its counterparts were equivalent (Table 4). This showed by the equivalence value that is below the maximum threshold (<1.5) and above the minimum threshold (> 0.5). LoC is acceptability threshold, either quantitatively or qualitatively, for adverse effects on the environment [19]. For field studies, EFSA suggests an effect size of 50% as a possible LoC value [6,20].

Table 3. Total dissolved solid, sugar content, total carotenoids, lycopene content, and β carotene of transgenic miraculin tomato plants and non-transgenic miraculin tomato.

Plant	Total dissolved solid (^{0}Brix)	Sugar content			Carotenoids (µg/g)	Lycopene (µg/g)	β-Carotene (µg/g)
		Glucose (%)	Fructose (%)	Sucrose (%)			
Transgenic	5.29 ± 0.20a	3.50 ± 0.22 a	2.60 ± 0.13 a	0.03 ± 0.002 a	14.13 ± 0.97 a	24.33 ± 1.72 a	13.95 ± 0.79 a
Non-Transgenic	5.14 ± 0.34a	3.60 ± 0.21 a	2.65 ± 0.11 a	0.03 ± 0.003 a	15.98 ± 1.07 a	29.16 ± 2.05 a	13.69 ± 0.63 a

Values with the same letter in the same column are not significantly different according to student's t-test. Data show mean values with standard error of the means (n = 80).



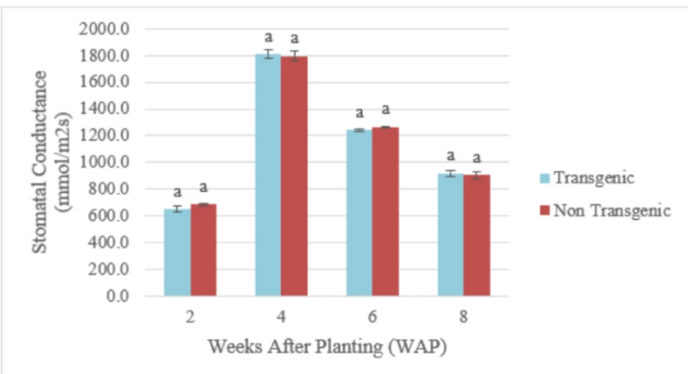


Figure 2. Chlorophyll content and stomatal conductance. Values with the same letter are not significantly

different according to student's t-test. Data show mean values with standard error of the means (n = 80).

This study is consistent with the previous research conducted by Carsono et al. [14]. The result shows that the miraculin transgenic tomato was equivalent to its non-transgenic counterpart. This indicates that the occurrence of unintended effects in miraculin transgenic tomato was not detected. The possible reason is because the miraculin gene is genetically stable. Genomic southern blot analysis of transgene confirms stable inheritance of single copy miraculin gene through multiple generations.The insertion of this gene into the tomato plant genome does not cause genome rearrangement which can result in phenotypic alterations [2]. In addition, the transgene might not affect cellular function of many traits or transcription factor, other regulatory proteins or molecules affecting multiple pathways. [7].

The result is also in line with other studies. Comparative field observations of miraculin transgenic tomato and its conventional counterpart have performed in Japan from 2018–2019. Traits evaluated in these field trials included plant morphology and growth characteristics. The statistical analysis over all sites revealed no statistically significant differences [21]. In the case of other environmental risk assessment procedures, such as weediness and invasiveness potential, the miraculin transgenic tomato was also equal to its counterpart. There is no evidence that the introduced miraculin gene by the genetic modification results in increased invasiveness and allelopathic compounds of tomato [22].

Table 4. Limit of concern traits.

No.	Characteristics	Equivalence value	Percentage difference	Note
1.	Plant height (0 WAP)	0.99	1%	Eq
2.	Plant height (2 WAP)	0.98	2%	Eq
3.	Plant height (4 WAP)	1.00	0%	Eq
4.	Plant height (6 WAP)	1.00	0%	Eq
5.	Plant height (8 WAP)	1.03	3%	Eq
6.	Stem diameter (0 WAP)	1.04	4%	Eq
7.	Stem diameter (2 WAP)	1.00	0%	Eq
8.	Stem diameter (4 WAP)	0.99	1%	Eq
9.	Stem diameter (6 WAP)	0.99	1%	Eq
10.	Stem diameter (8 WAP)	1.03	3%	Eq
11.	RGR (2-0 WAP)	1.00	0%	Eq
12.	RGR (4-2 WAP)	1.04	4%	Eq
13.	RGR (6-4 WAP)	1.00	0%	Eq
14.	RGR (8-6 WAP)	0.67	33%	Eq
15.	Days to 50% Flowering	1.00	0%	Eq
16.	Days to Maturity	1.00	0%	Eq
17.	Number of flowers per cluster	1.03	3%	Eq
18.	Number of fruits per cluster	1.07	7%	Eq
19.	Number of fruits per plant	1.03	3%	Eq
20.	Fruit weight	0.97	3%	Eq
21.	Fruit diameter	0.97	3%	Eq
22.	Harvest index	1.10	10%	Eq
23.	Total dissolved solid	1.03	3%	Eq
24.	Fructose content	1.06	6%	Eq

25.	Glucose content	0.98	2%	Eq
26.	Sucrose content	1.00	0%	Eq
27.	Total carotenoids	0.88	12%	Eq
28.	Lycopene content	0.83	17%	Eq
29.	β-carotene content	1.02	2%	Eq
30.	Chlorophyll content (2 WAP)	1.02	2%	Eq
31.	Chlorophyll content (4 WAP)	1.04	4%	Eq
32.	Chlorophyll content (6 WAP)	1.04	4%	Eq
33.	Chlorophyll content (8 WAP)	1.02	2%	Eq
34.	Stomatal conductance (2 WAP)	0.95	5%	Eq
35.	Stomatal conductance (4 WAP)	1.00	0%	Eq
36.	Stomatal conductance (6 WAP)	0.98	2%	Eq
37.	Stomatal conductance (8 WAP)	1.00	0%	Eq

Remarks: WAP = weeks after planting; RGR = relative growth rate; eq = equivalence.

The difference in characteristics between transgenic plants compared to conventional plants may occur due to in vitro culture of target tissue, such as callus, and possibly due to the insertion of transgenes. This process can cause alternation in plant genomic DNA including genetic variations (mutations), epigenetic variations, and the influence of regeneration techniques that result in somaclonal variations and gene expression due to insertional of the transgene [7,23]. This change is also due to the genetic and environmental interactions [24,25]. Environmental factors such as temperature, humidity, and rainfall affect the phenotype expression in transgenic plants[26]. In this research, there were changes in these characteristics of miraculin transgenic and non-transgenic tomato such as low average number of fruits per plant (14.30 and 13.90) and fruit diameter (30.99 and 31.81 mm). Tomato cv. Moneymaker has the average number of fruits per plant and fruit diameter which are 31.9 and 51.2 mm [27].

These characteristics still meet the principle of equivalence because the changes occur in uniform. During the experiment, the maximum day temperature is above the optimum temperature (21–29.5 ℃) [28] reached 32 ℃. High temperature can decrease pollination efficiency including pollen viability and tomato fruit production. High temperatures can reduce the rate of DNA synthesis and inhibit the chromosome condensation process which in turn affects the failure of tetrad development during the meiosis stage [29]

In this research, the maximum humidity reached 91%. The suitable humidity level for growing tomato plants is approximately 50–70%. Tomato plants are sensitive to high humidity especially during the generative phase. This condition has impact on decreasing fruit quantity and quality. High humidity can reduce the rate of transpiration which results in the loss of plant cell turgor [28].

The high rainfall during the experiment was also a limiting factor for the growth and development of tomato. High rainfall can decrease the number of fruits due to an increase in the percentage of shed flowers by around 50% [30]. The high humid conditions due to high rainfall are suitable for the development of bacterial wilt disease. Ralstonia solanacearum bacteria thrive in soil during the rainy season [31]. These bacteria invade plants through the xylem vessels in the roots and produce exopolysaccharides (EPSs) which can inhibit water transportation from the roots to all plant tissues. This causes the photosynthesis process to be interrupted and the plant withers [32]. This disruption causes the unoptimal size, weight, and a number of fruits [33] and reduces the fruit dry weight and stover up to 26.9–38.2% [34].

4. Conclusions

In this study, the comparative analysis of miraculin transgenic tomato cv. Moneymaker and its origin showed no significant difference in all agronomic, compositional, and physiological traits. This indicates that the miraculin transgenic and non-transgenic tomato were equivalent. Any unintended effects were not detected. Further research is required to assess the miraculin transgenic tomato in multi-locations of field trials.

Acknowledgments

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Essential oils rich in pulegone for insecticide purpose against legume bruchus species: Case of Ziziphora hispanica L. and Mentha pulegium L.

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A correction on

Essential oils rich in pulegone for insecticide purpose against legume bruchus species: Case of Ziziphora hispanica L. and Mentha pulegium L.

by Tarik Ainane, Fatouma Mohamed Abdoul-Latif, Asmae Baghouz, Zineb El Montassir, Wissal Attahar, Ayoub Ainane and Angelo Maria Giuffrè. AIMS Agriculture and Food, 2023, 8(1): 105–118. DOI: 10.3934/agrfood.2023005

The authors would like to make the following corrections to the published paper [1]. The changes are as follows:

Adding co-author Asmae Baghouz and affiliation 3:

From

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To

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Updating Figures 1 and 2 for more clarity:

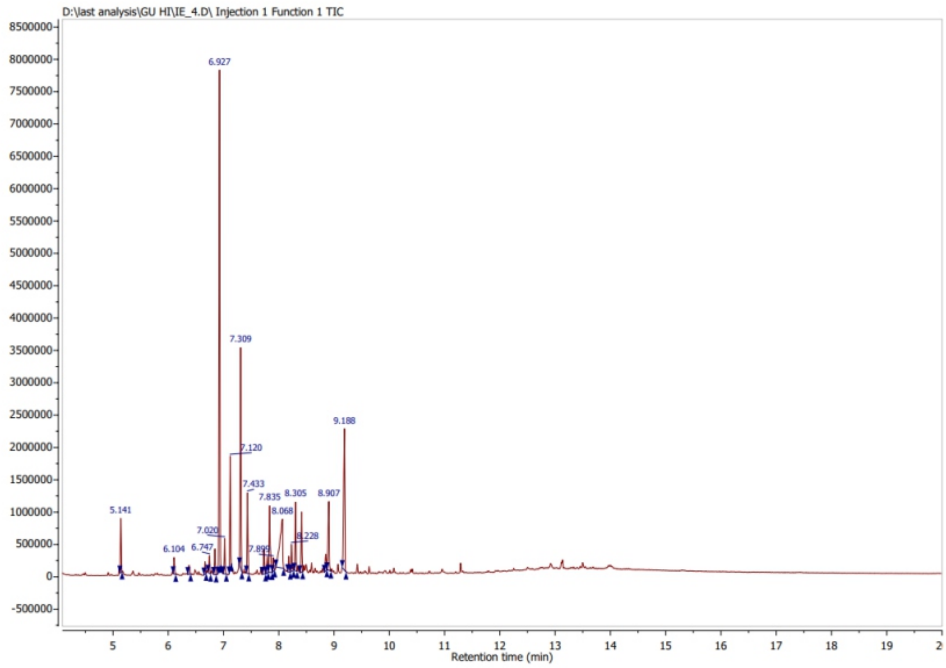

Figure 1. Chromatogram of the Ziziphora hispanica essential oil.

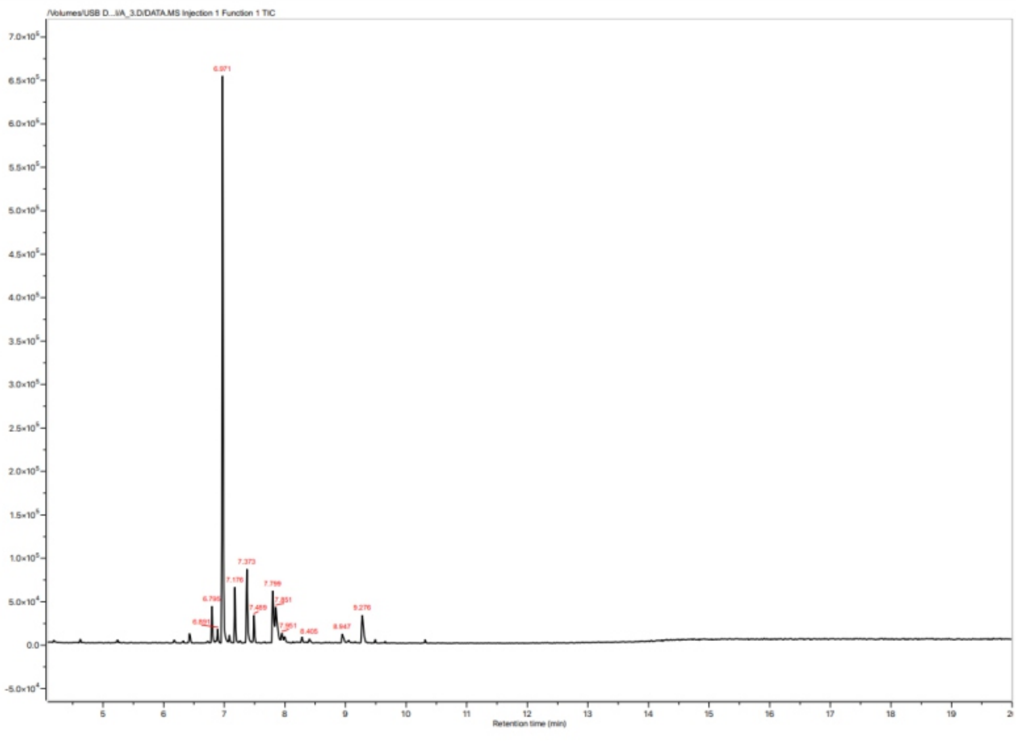

Figure 2. Chromatogram of the Mentha pulegium essential oil.

The added co-author is the original author of Figures 1 and 2, and he provides a clearer version of the chromatograms. These changes have no material impact on the conclusion of this article. The original manuscript will be updated [1]. We apologize for any inconvenience caused to our readers by this change.

Conflict of interest

The authors declare that they have no competing interests.

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1. Ainane T, Abdoul-Latif FM, Baghouz A, et al. Essential oils rich in pulegone for insecticide purpose against legume bruchus species: Case of Ziziphora hispanica L. and Mentha pulegium L. (2023)AIMS Agric Food 8: 105–118. https://doi.org/10.3934/agrfood.2023005

Innovation in agriculture and the agri-food chain: Some insights

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Research and innovation in sustainable food systems is a focus of public policy, such as that of the European Union. It has become evident that business-as-usual market patterns are not compatible with the growing demands of environmental and social sustainability.

Population growth, urbanization, migration, resource scarcity, climate change, and environmental degradation [1] are creating increasingly urgent and complex challenges.

Therefore, agri-food systems, research, and innovation need to combine a new production and consumption paradigm, resulting in new sustainable, resilient, inclusive, and healthy market opportunities to achieve the UN Sustainable Development Goals and to realize the policy objectives of the EU Farm-to-Fork strategy and Green Deal.

This Editorial aims to report a critical reading of some examples of innovation in the agri-food sector, with the objective of concluding the discussion opened by the ten research articles published in the AIMS Agriculture and Food in the Special Issue: Innovation in agriculture and the agri-food chain.

Several studies demonstrated the importance of product innovation to increase visibility, accessibility, consumers' loyalty and purchasing intention. Stakeholders in the agri-food chain can act at various levels to implement innovative solutions in the production process or during product development/renewal. Process/product improvement strategies must include market analysis considering, among other things, the study of the consumer in order to successfully define product positioning in the minds of potential buyers of the innovative product. Product innovation strategies include those that renew the image of the product itself, which can be transformed from "conventional" to "certified", "sustainable", and "safe".

At the supply chain level, a sustainable production system, perhaps certified, represents a product enhancement factor to which consumers show increasing attention when choosing food products. However, the inclusion of this choice attribute is product/specific: as shown in Merlino et. al [2], for example, product characteristics attributable to the environmental sustainability dimension carry less weight during the assessment of product quality than convenience and safety attributes in ready-to-eat products, such as fresh-cut salads. On the contrary, product sustainability, encompassed in its social, economic and environmental dimensions, ascribable to traditional and/or extensive food production systems, becomes a discriminating factor in the choice of 'mountain' or 'local' products, as e.g. Brun et al. [3] demonstrated in the case of honey. At the same time, the assessment of the sustainability of the supply chain, supported by technological innovation and the circular economy, is considered an important factor evaluated by the various countries of the European Union both in traditional supply chains and, above all, in novel food supply chains, in order to assess their feasibility and durability in the long term [4].

From another perspective, the transition to an innovative supply chain can be interpreted as the inclusion of advanced practices and management changes made in order to increase food security and health [5,6]. In fact, as described by [7,8] in traditional traceability management systems often involve considerable bureaucratic, economic and managerial effort on the part of companies, especially small and medium-sized enterprises, which make up most of the food sector. The same authors, moreover, starting from a thorough literature review, developed a low-cost open-source traceability system focused on food safety

and quality. In order to encourage the adoption of inexpensive and managementsustainable traceability systems with ultimate benefits for producers, retailers and consumers [7,8].
In the latter projection, the increasing of traceability efficiency systems [6], also using technological approach as the blockchain system [9] can favor the management of traceability systems also for small and medium-sized enterprises and increase the transparency of the supply chain in the eyes of consumers. In particular, Osei et al. [9] identified the novelty of the technology, supply chain characteristics, open data issues, cost-benefit analysis, and role of public stakeholders has the main factors affection the Blockchain technology adoption, in particular in fresh-food supply chain.
Product innovation, on the other hand, besides involving intrinsic product characteristics, often includes a decision-making process about the packaging image. Packaging is the factor most involved in communication strategies as it is the part of the product that most directly interacts with the consumer during the choice process. However, some sectors are characterized by anonymous, standardized packaging that can hardly compete with nascent competitors: cow's milk vs. vegetable drinks, for example. Despite the fact that the cow's milk sector has been going through a critical moment for years, both in terms of consumption and production, packaging could nevertheless represent an appealing and differentiating tool for consumers. In fact, the study by Merlino et al. [10]showed how consumers are willing to buy and spend a premium price for innovative, more environmentally sustainable packaging, but also characterized by a more convenient and practical opening system. An innovative packaging, in the case of craft beer [11], was evaluated as a tool for product novelty by assessing the acceptability of the use of the can by a sample of consumers. In conclusion, this research shows how a target of consumers accepts the use of the can for craft beers. This work showed how the can represents a key positive element for innovation as, not only could it be accepted by consumers, but it can accommodate attractive graphics, also providing logistical advantages over the traditional glass.
These studies thus demonstrated the impact of process and product innovation on production efficiency, safety, healthiness and product quality. Innovation, undertaken in its various perspectives, can therefore represent a tool for growth and improvement of company efficiency, thus becoming a tool for enhancing and differentiating products on the market.

Conflict of interests

The authors declare no conflict of interest.

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