



## Investigation of amaranth production constraints and pest infestation reduction by basil intercropping

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### ABSTRACT

Amaranth (*Amaranthus cruentus* L.) is the most consumed leaf vegetable in Benin. A study carried out in south Benin have shown that the production of this vegetable is severely limited by insect pest pressure. The present study aimed to identify the major constraint limiting amaranth production in Ségbana municipality, located in the north of Benin and proposed sustainable agroecological solutions to farmers. Thus, a survey was conducted among 150 farmers in three villages of Ségbana (Lougou, Sokotindji and Piami) through well-structured questionnaires to know the major constraint limiting amaranth production. Agroecological methods for managing pests of this vegetable were offered to farmers through an experimental trial consisting of a Fisher block with four treatments and five replications conducted in Sokoundji village. The control treatment (To) consisted of amaranth in pure culture; the treatment (T1), amaranth plants surrounded by basil plants; (T2) rows of amaranth alternated with basil rows and (T3), amaranth plants alternated with basil plants in all directions. Every 5 days, 5 plants were randomly selected per treatment and the numbers of *S. recurvalis* and *P. basalis* were counted. Pest damage and yield per treatment were also assessed. Almost all farmers surveyed (100%) reported pest attacks as the major constraint limiting amaranth production. The results obtained on the abundance and the herbivory rate showed powerful negative effects ( $Df = 3$ ;  $P < 0.0001$ ) of the different treatments. Moreover, the treatment T2 (amaranth plants alternated with basil rows) gave the highest yield (1.25 t/ha of fresh leaves) and differed significantly from the other treatments ( $Df = 3$ ;  $p = 0.039$ ). The association basil – amaranth reduced the abundance of *P. basalis* and *S. recurvalis* and also improved the amaranth productivity with  $LER = 1.16$ . The association basil – amaranth is more beneficial than pure cultures because it hosts less of the insect pests studied, provides higher yields and makes rational use of the growing space.

### 1. Introduction

Agriculture is one of the main sectors contributing to economic development. In West Africa, and specifically in Benin, it employs more than 42.36% of the active population, contributes to 75% of export earnings and provides around 70% of jobs [1,2]. The economic contribution of agriculture is based on a number of activities; among these, market gardening occupies a very important place and contributes significantly to food sovereignty, the fight against poverty and to family income [3,4,5]. In Benin current context, market gardening has become

one of the priority sectors to be promoted in the "Plan Stratégique pour la Relance du Secteur Agricole (PSRSA)" and in the "Plan Stratégique de Développement du Secteur Agricole (PSDSA)." It employs about 4% of the active population, or 60,000 jobs [5]. Actual data indicate that the margin generated by market gardening can reach 16.395 million CFA francs/ha/year, or 4.31 billion on an area of 263 ha exploited in 2000 [6]. The growing importance given to vegetable cultivation is due to the primordial role it plays in the economies of most of these African countries [7]. Indeed, vegetables are an important component of daily diets, and sources of income, particularly in urban and peri-urban areas

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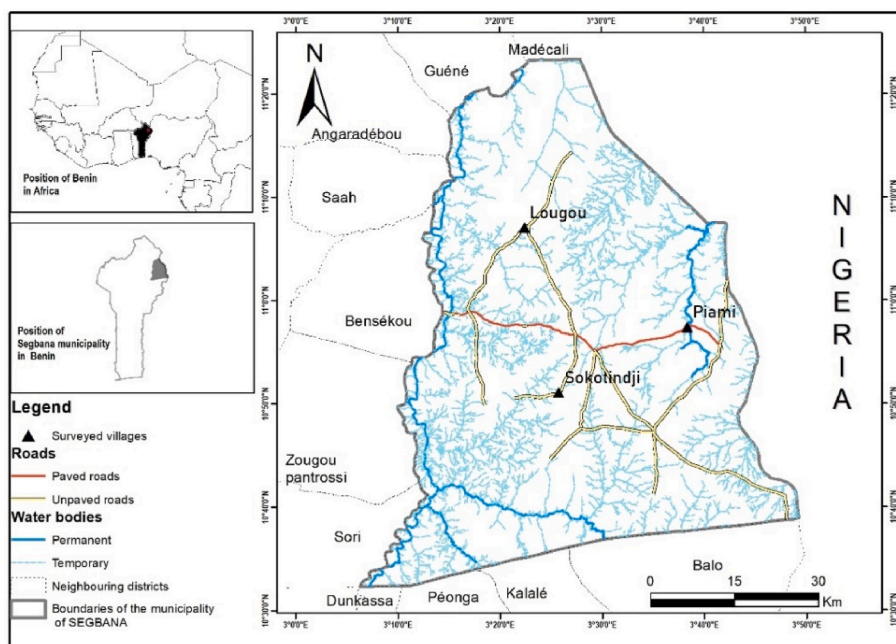


Fig. 1. Survey site.

[4]. They can be grouped according to the part of the plant that is consumed and/or sold. Thus, we find leafy vegetables (amaranth, nightshade, cabbage), fruiting vegetables (tomato, chilli) and root vegetables (carrot) which are all important sources of plant proteins, vitamins, and trace elements for human and animal nutrition [8]. Amaranth (*Amaranthus* spp) is a traditional vegetable whose leaves and seeds are consumed in various forms [9,10]. In Benin, there are several varieties, but *Amaranthus cruentus* is the most cultivated and consumed, because leaves are rich in protein, vitamin C, beta-carotene, iron and calcium [11–13].

However, its production is limited by several challenges including abiotic and biotic constraints. Climate change is not only creating favourable conditions for the development of pests (leafminers, mites, whiteflies, aphids, thrips, moths, bugs) and diseases, but is also altering their modes of transmission [1,14,15]. According to Ref. [4], the damage caused by these pests can be estimated at 100% yield loss. Attempts to control these pests consist of the frequent and unreasoned use of synthetic chemical insecticides that can lead to chemical residues on fruits and vegetables, biological imbalance, environmental contamination, intoxication of people and animals, as well as the appearance of pest resistance, the elimination of useful entomofauna, in particular parasitoids [4,16–20]. However, chemical control is not always effective in the short or medium term due to the use of unsuitable, poorly dosed or overused products.

Environmental-friendly agricultural practices have retained agronomists attention these latest years to solve the problems. An interesting approach in this respect is the use of pesticide plants [21–23]. The biocidal activities of various plant families used as extracts, essential oils or repellent odor sources have been demonstrated on various pests [24, 25].

Certain aromatic plants, according to their particular odors, can keep away or eliminate harmful insects. Among these plants are those of the genus *Ocimum* which are plants of the Lamiaceae family, rich in aromatic essential oils. They are collectively known as the “basils” and are represented by more than 150 species cultivated and distributed throughout the tropical and temperate regions [26]. Different basil types are commonly used, including holy basil (*O. sanctum* L.), sweet or Thai basil (*O. basilicum* L.), lemon basil (*O. citriodorum* L.), and tree basil (*O. gratissimum* L.) [27,28]. *Ocimum gratissimum* is an erect hairy

aromatic herb used as relief for blocked nose [29], delicious teas [30], mosquito repellence [31] and treatment of snake bites [32]. A literature survey has revealed that *O. gratissimum* has anti-microbial activity against both gram positive and gram-negative bacteria [33], anti-fungal effect against *Alternaria alternata*, *Alternaria tenuissima*, TZ10.2.2 and TZ8.2.2 [34], insecticidal and repellent effects against various insect species [35–39]. Several studies have shown a repellent effect on pests when the crop was intercropped with *Ocimum* species [22,40–42].

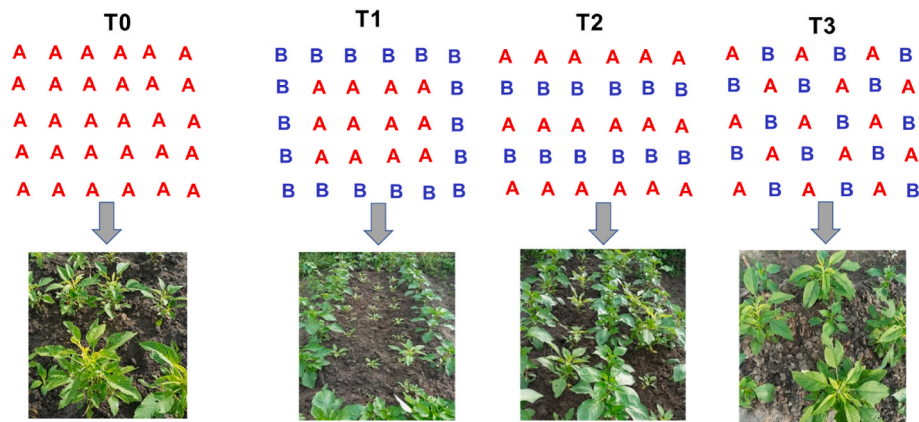
To increase the production of amaranth, the Government of Benin has set up the "Projet d'Appui au Développement du Maraîchage Résilient aux Changements Climatiques et d'Amélioration des Revenus des Exploitants" (PADCMA RCARE) in the Segbana municipality to support small-scale producers, mainly women, to improve their income. A recent study has shown *Spoladea recurvalis* Fabricius and *Psara basalis* Walker as the main pests encountered on amaranth in south Benin [43]. The main goal of this study was to identify the real constraints limiting amaranth production in north Benin through a survey using well-structured questionnaires, and then to propose a sustainable agroecological approach to control two main pests of amaranth, *S. recurvalis* and *P. basalis*.

## 2. Materiel and methods

### 2.1. First step: survey design and data description

The municipality of Segbana (north Benin) is located in the Alibori department and bounded: to the north by Malanville municipality, to the south by Kalale municipality, to the east by the Federal Republic of Nigeria and to the west by Kandi and Gogounou municipalities (Fig. 1). Segbana is characterized by a monomodal rainy pattern, with rains occurring May to October with annual rainfall generally between 800 and 1200 mm<sup>3</sup> [44] and dry season from October to May. The monthly relative humidity across the country ranges from a minimum of 44% to a maximum of 99%.

In 2018, a sampling visit were conducted from 15th July to 30th August in Segbana municipality. During the survey, three (3) towns were visited: Lougou, Sokotindji and Piami. The survey was conducted among 150 farmers. The choice of farmers was mainly based on the importance of amaranth production. A total of 21 questions were asked



**Fig. 2.** Schematic and picture illustration of the different treatments

A: amaranth, B: basil; T0: control treatment with amaranth in pure culture; T1: amaranth plants bordered by basil plants; T2: amaranth plants alternated with basil rows; T3: amaranth plants alternated with basil plants in all directions.

and the interviews took on average 15 min per farmer. After obtaining information on the social situation, each farmer was asked about the amaranth production, the main pests causing damage to it. They were also asked about the major constraints encountered in amaranth production and the solutions applied.

## 2.2. Second step: bioassay

### 2.2.1. Plant material

*Ocimum gratissimum* L. (basil) and *Amaranthus cruentus* L. were used in this study. The seeds were purchased from approved shops in Cotonou.

### 2.2.2. Experimental design

The experimental design (Fig. 2) used in this study was the Fisher block. Four subplots per bloc measuring 6 m × 1.2 m were made. The amaranth nursery was carried out one week after sowing the basil plants. The nurseries of both crops were realized directly on the ground. The portion of land delimited of that was disinfected with hot water to kill any pests hidden in the ground. After 24 h, the ground was well plowed and mixed with compost; the beds were then dressed, watered and the sowing lines were drawn using a piece of wood, furrows spaced with 1 cm of deep. For sowing, amaranth and basil seeds were mixed with a little fine sand and the blend was spread in the furrows. Next, the nursery was covered with a thin layer of sand, watered it and cover it with straws then treated with fungicide Topsin M (10 g mixed in 11L of water). Three days later for amaranth and ten for basil, the straws were removed and replaced by a shadehouse. The young seedlings with 3-4 well-developed leaves were transplanted three weeks later for amaranth (with a density 20 cm × 20 cm) and four weeks for basil (with a density 30 cm × 30 cm) on well-dressed beds. One week after transplanting, plants were fertilized with a mixture of manure, chicken droppings and cow purse all at the rate of 10 tones/hectare, i.e. 7.2 kg/subplot. Because of the poorly state of soil, in addition to organic fertilizer, Urea and NPK were also added the second week after transplanting at 75 kg/ha (i.e. 10 g/m<sup>2</sup>). The basil plants were transplanted 2 weeks before the amaranth plants. A reasonable quantity of water was used to daily water the amaranth plants manually with watering can morning at 7 a.m. and afternoon at 5 pm.

During the bioassay, no chemical was applied. Four treatments were tested: T0 (Control treatment with amaranth in pure culture); T1 (amaranth plants bordered by basil plants); T2 (amaranth plants alternated with basil rows); T3 (amaranth plants alternated with basil plants in all directions) (Fig. 2).

## 2.3. Data collection

Sampling started 14 days after transplanting and every 5 days, 5 plants were randomly selected per treatment and the numbers of *S. recurvalis* and *P. basalis* were counted and recorded. The pest damages were also evaluated. The mean leaf damage index was identified following [45] ranking: 0 = no damage; 1 = little damage (pinholes, and/or small holes, small leaf edge parts eaten, shot holes); 2 = medium damage (some larger holes and/or larger leaf edge areas eaten); 3 = heavy damage (many larger holes and/or larger leaf edge areas eaten) and 4 = total damage (destroyed, non-functional leaves). The harvest started 21 days after transplanting for amaranth and 30 days for basil. At harvest, the plants have been cut, counted and classified into three

categories: plants totally attacked, plants totally healthy and plants partially damaged (damaged but part of which is still marketable). Leaves were then weighed using the Weiheng Brand Scale 10 Kg reach with 5 g. The Land Equivalent Ratio (LER) was also calculated using the formula  $LER = \sum (Yp_i/Ym_i)$ , where  $Yp$  is the yield of each crop or variety in the intercrop or polyculture, and  $Ym$  is the yield of each crop or variety in the sole crop or monoculture. For each crop (i) a ratio is calculated to determine the partial LER, then the partial LERs are summed to give the total LER for the intercrop [46–48]. An LER value of 1.0, indicates no difference in yield between the intercrop and the collection of monocultures [48–50]. Any Value greater than 1.0 indicates a yield advantage for intercrop. The results obtained were estimated per hectare.

## 2.4. Data analysis

The data from the survey were analyzed by using analysis of variance followed by the Bonferroni test according to the losses in production estimated by the farmers. We determined the pest abundances by calculating the number of larvae collected per treatment. Generalized Linear Models (GLMs) with the Poisson family were used to determine the variation in the pest abundances and vegetable yields according to the different crop associations (treatments). GLMs with the Binomial family were used to determine the variation in the herbivory rate according to the different treatments. To test the direct and indirect interactions between the crop associations and the pest abundances/herbivory rate, we used Structural Equation Modeling (SEM) with the 'lavaan' package [51]. Collected data were analyzed using the software R version 3.4.2 [52].

**Table 1**  
Identifications and prioritization of constraints.

N°	Constraints reported	Responds
<b>Production</b>		
1	No knowledge in pests control	100%
2	No knowledge in technical route	40%
3	Insufficient technical support	33.33%
<b>Conservation</b>		
4	Lack of storage store	20%
5	Lack of conservation equipment	25%
<b>Marketing</b>		
5	insufficient disposal market	60%



Fig. 3. *Spoladea recurvalis* damages on amaranth leaves.

### 3. Results

#### 3.1. Identification and prioritization of constraints

The constraints mentioned by farmers have been prioritized and ranked into constraints of production, conservation and marketing. Almost all of the farmers (100%) reported the pest attacks (Table 1) as being the major constraint limiting the amaranth production, while 40% and 33.33% respectively reported the lack of technical route and support. In addition, 20% and 25% respectively estimated that the lack of storage warehouse and the lack of conservation equipment are also limiting factors in amaranth production. The insufficiency of the market was also reported by a large number of farmers (60%) (Table 1).

#### 3.2. Pests abundance and damage on amaranth

The Fig. 3, Fig. 4 (a and b) and Fig. 5 showed respectively the relative

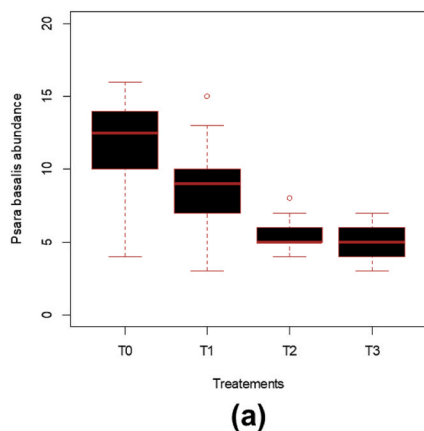


Fig. 4. Effect of different associations on *P. basalis* (a) and *S. recurvalis* (b) abundances

T0: control treatment with amaranth in pure culture; T1: amaranth plants bordered by basil plants; T2: amaranth plants alternated with basil rows; T3: amaranth plants alternated with basil plants in all directions.

abundance and pest damage of *P. basalis* and *S. recurvalis* of the different vegetable associations. The abundances of the two species were higher on the T0 and T1 treatments Fig. 4 (a and b). Moreover, Structural

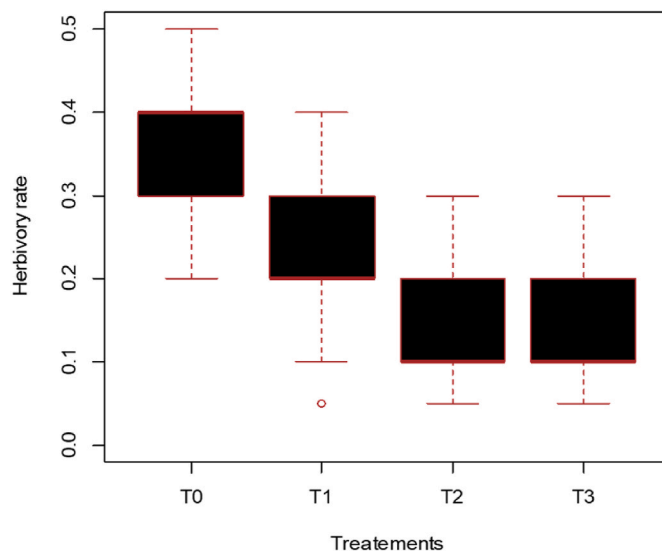


Fig. 5. Effect of different associations on pest damages

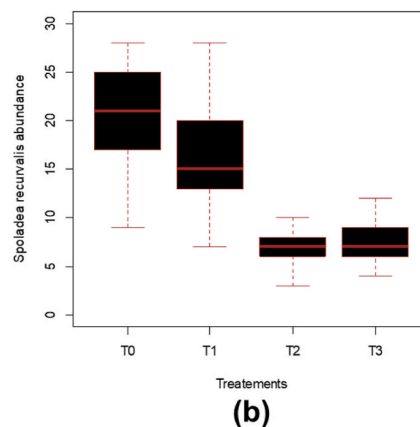
T0: control treatment with amaranth in pure culture; T1: amaranth plants bordered by basil plants; T2: amaranth plants alternated with basil rows; T3: amaranth plants alternated with basil plants in all directions.

**Table 2**

Abundance relative of *P. basalis* and *S. recurvalis* on amaranth.

Treatments	Df	SE	t-value	Pr(> t )
<b><i>Psara basalis</i></b>				
Intercept	116	0.4260	26.763	<0.0001***
T1	116	0.6024	-4.427	<0.0001***
T2	116	0.6024	-9.684	<0.0001***
T3	116	0.6024	-10.56	<0.0001***
<b><i>Spoladea recurvalis</i></b>				
Intercept	116	0.7458	26.81	<0.0001***
T1	116	1.0547	-3.856	0.00019 ***
T2	116	1.0547	-12.262	<0.0001***
T3	116	1.0547	-12.262	<0.0001***

T1: amaranth plants bordered by basil plants; T2: amaranth plants alternated with basil rows; T3: amaranth plants alternated with basil plants in all directions.





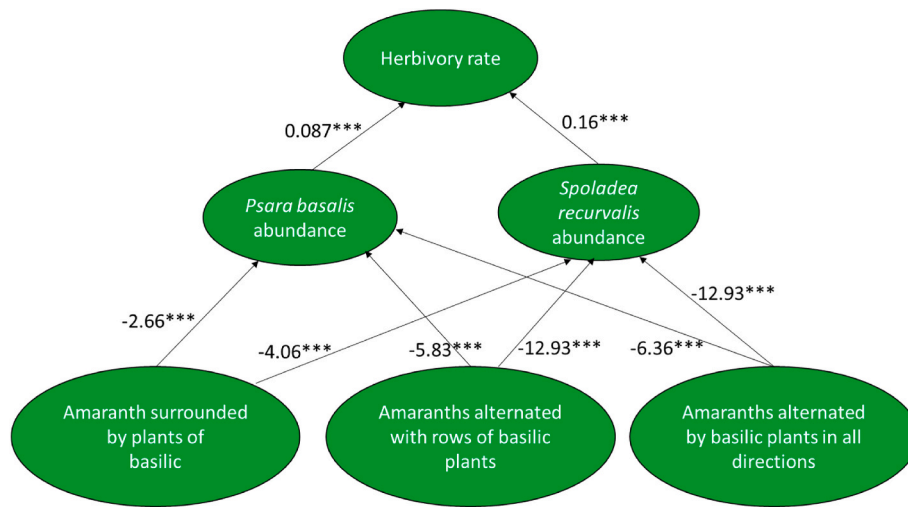


Fig. 6. Structural Equation Models (SEM) showing the relationship between the different configurations of vegetable associations and the abundances of the two pests and then the relationship between the abundances of the pests and the herbivory rate.

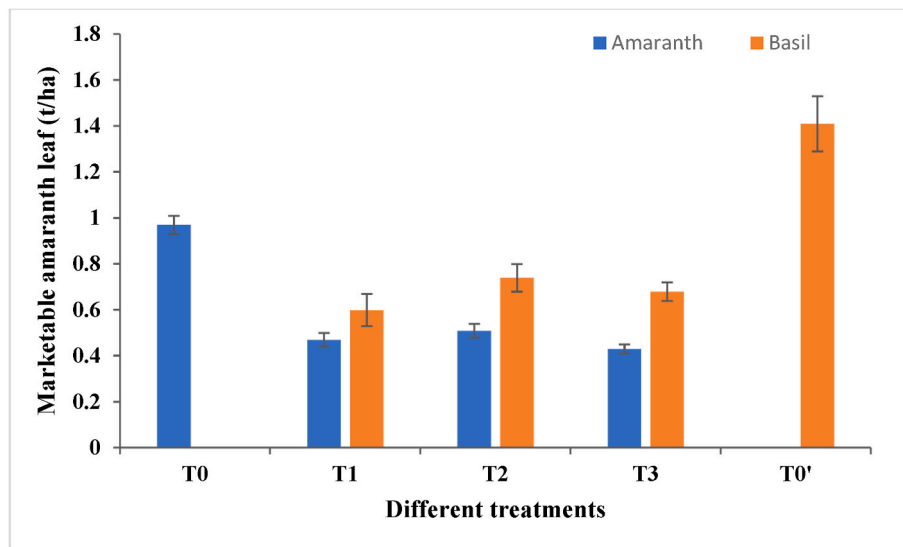


Fig. 7. Effect of different associations on vegetable yields

T0: control treatment with amaranth in pure culture; T1: amaranth plants bordered by basil plants; T2: amaranth plants alternated with basil rows; T3: amaranth plants alternated with basil plants in all directions.

Equation Models showed a significant difference between the different types of association (Df = 3; P < 0.0001) that exerted a powerful negative effect on the pest abundance and damage of *P. basalis* and *S. recurvalis* (Table 2) (see Fig. 5).

### 3.3. Relationship between pest abundance and pest damage

Fig. 6 showed on the one hand the relationship between the different associations of vegetables and the relative abundances of the two pests and on the other hand the relationship between the abundances of the pests and the rate of herbivory. Structural Equation Models showed significant effects of the abundance of *P. basalis* (Df = 3; P < 0.0001) and *S. recurvalis* (Df = 3; P < 0.0001) on herbivory rate. In addition, the abundance of pests has significant positive effects but is weak on the herbivory rate (Fig. 6).

### 3.4. Effects of different associations on the amaranth productivity

Fig. 7 showed the effect of different cropping systems on the

amaranth yield. The yield varied from  $0.97 \pm 0.04$  (Control treatment) to  $1.25 \pm 0.09$  (T2: amaranth plants alternated with basil rows) (Table 3). The treatment T2 (amaranth plants alternated with basil rows) showed the highest yield ( $1.25 \pm 0.09$ ) followed by the T3 (amaranth plants alternated with basil plants in all directions) ( $1.11 \pm 0.06$ ) and T1 treatments (amaranth plants bordered by basil plants) ( $1.07 \pm 0.1$ ) (Fig. 7). The Generalized Linear Models (GLMs) showed a significant difference in vegetable yields across the different associations (Df = 3; P = 0.039).

## 4. Discussion

This study carried out in northern Benin have showed constraints relative to the amaranth production and the use of environmental-friendly agricultural practices such as approach in this respect like plants pesticide to control pests [21–23]. Our results showed there are many factors that hinder the production of this vegetable. As limiting constraints, few farmers reported the lack of technical route and support while other estimated that the lack of storage warehouse and the

**Table 3**  
Yield agronomic evaluation.

Treatments	Yield (t/ha)			Land Equivalent Ratio (LER)		
	Amaranth	Basil	Total	Amaranth	Basil	Total
T0	0.97 ± 0.04	–	0.97 ± 0.04	1	–	–
T0'	–	1.41 ± 0.12	1.41 ± 0.12	–	1	–
T1	0.47 ± 0.03	0.6 ± 0.07	1.07 ± 0.1	0.49	0.41	0.90
T2	0.51 ± 0.03	0.74 ± 0.06	1.25 ± 0.09	0.65	0.51	1.16
T3	0.43 ± 0.02	0.68 ± 0.04	1.11 ± 0.06	0.44	0.47	0.90
Df	3					
P	0.039					

T0: Control treatment with amaranth in pure culture; T0': Control treatment with basil in pure culture T1: amaranth plants bordered by basil plants; T2: amaranth plants alternated with basil rows; T3: amaranth plants alternated with basil plants in all directions.

conservation equipment follow by insufficiency of the market. Almost all of them (100%) reported the pest attacks as being the major constraint in the amaranth production. Our results confirms those of [43,53,54] who have shown pest attack as the important factor limiting the production of this vegetable. Apart from pests attack [53], reported certain constraints related to production, processing, distribution and marketing who hinder its development and maintain it in the status of a neglected and underused species. It is therefore necessary to develop this production and diversify amaranth products for national and international markets, knowing that it is a very well vegetable appreciated in Beninese dishes.

Environmental-friendly agricultural practices like crop association appears to be an interesting approach [21–23] that offer major potential in market gardening, in reducing the use of chemical products while maintaining a satisfactory level of yield in terms of quality and quantity [55]. In the second part of this study, we evaluated the effect of basil/amaranth to control *P. basalis* and *S. recurvalis*, two major pests limiting amaranth production [54]. The results showed that treatments T2 (amaranth plants alternated with basil rows) and T3 (amaranth plants alternated with basil plants in all directions) harbored fewer pests overall than T1 (amaranth plants bordered by basil plants) and T0 (Control treatment with amaranth in pure culture) treatments. Indeed, several studies have shown that cabbage plots in association with basil plants show less infestation by *Spodoptera littoralis* Boisid, *Plutella xylostella* Linnaeus and *Hellula undalis* Fabricius than those of cabbage in pure culture [22,56]. Moreover [57], reported less damage of *H. undalis* to cabbage grown in association with pepper or onion. Similar results were also reported by Ref. [58] who found a low density of mites *Polyphagotarsonemus latus* Banks in the gboma field by using an aqueous extract of basil, Neem oil and Sunpyrifos. Other study carried out by Ref. [59] in the cropping system gboma/basil showed also low density of aphids, *Selepa docilis*, *Bemisia tabaci* and mealybugs. Our result could also be explained by the insecticidal and repellent properties of basil [34–39]. Indeed, the essential oil of the basil was principally composed by the aromatic ether estragol (74.0%) and the terpene with alcohol group linalool (17.8%), which are known to have repellent and toxic activities against stored product insect [60]. The repellent effect of aromatic plants on insects is generally attributed to their volatile organic compounds (VOCs) shown to disturb the oviposition of *P. xylostella* and *Pieris brassicae* Linnaeus on cabbage plants in association with clover [61]. According to the same authors, this disturbance is much more linked to the presence of repellent compounds than to the physical appearance of the clover. In fact, volatile organic compounds (VOCs) emitted by aromatic plants greatly influence the process of localization of host plants by pests [62,63]. The genus *Ocimum*, has been also investigated with regard to its insecticidal properties against diverse

insect pests [64–67].

These properties of the genus *Ocimum* have been demonstrated on various arthropods [68–70]. In fact, the biocidal effect of these plant species to control pests would be linked to phenol, which is a phytochemical compound present in the plant [71,72]. The low infestation of the insect pest population would therefore be linked to these egg-laying disturbances and to the phenol [73]. Thus, the volatile compounds emitted by basil plants would have disturbed the oviposition of *P. basalis* and *S. recurvalis* on amaranth plants. This could then explain on the one hand the low numbers of pests recorded in treatments T2 (amaranth plants alternated with basil rows) and T3 (amaranth plants alternated with basil plants in all directions). On the other hand, the activity of the natural enemies of these pests could also influence. Indeed, in pear orchards associated with *O. basilicum*, [74] demonstrated that *O. basilicum* emits volatile substances attractive to auxiliary insects. Thus, basil would have attracted the natural enemies of these pests in the associated crop plots. These natural enemies would have contributed to reduce the number of pests in plots of associated amaranth crops. The so-called natural enemy hypothesis of [75,76] that natural regulation by parasitism, predation, etc. is more effective in intercropping systems than in pure cropping systems is verified.

The best yields were obtained on T2 (amaranth plants alternated with basil rows) and T3 (amaranth plants alternated with basil plants in all directions) while the lowest were on T0 (Control treatment with amaranth in pure culture) and T1 (amaranth plants bordered by basil plants). The Structural Equation Models showed significant negative effects of pest abundances on intercropping but the effects are weaker for T2 and T3 treatments. This confirms the hypothesis that *Ocimum* plants secrete volatile substances that repel pests [77,78], so crop associations would have harbored natural enemies [79]. By these SEM, we understood that the two pests increase the rate of herbivory but not more important than in monoculture. These results confirm the insecticidal and biocidal properties of basil since the treatments that have been the most effective on pest populations are also those that have induced the best yields of amaranth.

The LER in the T2 (amaranth plants alternated with basil rows) = 1.16 is greater than 1. This means that the association amaranth plants alternated with basil rows have a potential interest in rationalizing the cultivable space. The amaranth plants alternated with basil rows (T2) could save 16% of the area compared to the pure culture of basil and amaranth. Our results are similar to those obtained by Refs. [80,81] on the cowpea/maize association, [82] on the cabbage/basil association and [59] on gboma/basil. According to these authors, the LER obtained were respectively 1.46, 1.07, 1.2, 1.11 et 1.40. The amaranth/basil cropping association would be a cropping system that allows better space management in the of increasing population context with consequent pressure on land resources.

## 5. Conclusions

From this study, we can conclude that the amaranth production is mainly limited by pest attacks and others constraints relative to the production, conservation and marketing. As agroecological practice to control *S. recurvalis* and *P. basalis*, the amaranth plants alternated with basil rows are the best cropping system. This spatial arrangement of these two vegetable crops provides a high yield of amaranth and shelters natural enemies for their conservation and the natural regulation of vegetable pests. We therefore suggest to continue investigation of other important vegetables more sensitive to arthropod pests in order to explore the potential of basil to really control these pests of vegetable crops.

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### Declaration of competing interest

The authors declare that they have no conflict of interest.

### Data availability

The authors do not have permission to share data.

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