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Full Length Research Paper

Pre-inoculation of soybean seeds: Effects on survival of *Bradyrhizobium elkanii*, nodulation and crop yield

Itacir Eloi Sandini[1*], Rafael Brugnera Belani[2], Margarete Kimie Falbo[3], Fabiano Pacentchuk[1] and Jaqueline Huzar-Novakowiski[4]

[1]Department of Agronomy, Universidade Estadual do Centro-Oeste (UNICENTRO), Rua Simeão Camargo Varela de Sá, 03, Vila Carli, 85040-080, Guarapuava, Paraná, Brazil.
[2]BASF S/A, Alameda Adélia Salvador Bernardo, 243, 13917-196, Jaguariúna, São Paulo, Brazil.
[3]Department of Veterinary Medicine, Universidade Estadual do Centro-Oeste (UNICENTRO), Rua Simeão Camargo Varela de Sá, 03, Vila Carli, 85040-080, Guarapuava, Paraná, Brazil.
[4]College of Agronomy and Veterinary Medicine – FAMV, Universidade de Passo Fundo (UPF), Avenida Brasil Leste, 285, São José, 99052-900, Passo Fundo, Rio Grande do Sul, Brazil.

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Pre-inoculation of soybean seeds with *Bradyrhizobium* spp. associated with polymers could be an alternative to the conventional inoculation performed at the day of sowing. The objectives of this study were i) to determine whether the HiCoat® technology (peat inoculant + liquid inoculant + polymer) allows the survival of *B. elkanii* after storage of the inoculated seeds, and ii) to evaluate whether the pre-inoculation and seed treatment reduces the physiological quality of seed and soybean yield. Soybean seeds were treated with different fungicides and insecticides associated with pre-inoculation using HiCoat® and the seeds were stored at room temperature. The survival of *B. elkanii* was reduced over time with standard inoculation, however there was above 2 x 10^6 colony-forming units of *B. elkanii* per seed at 60 days after inoculation with HiCoat®. Seed germination, number of nodules per plant, mass of nodules, yield, number of grains per plant and number of pods per plants were not significantly affected by the pre-inoculation and seed treatments. Our findings indicated that the fungicide and insecticide associated with HiCoat® can be used to treat and to pre-inoculate seeds that can be stored for up to 71 days without compromising the nodulation of plants and soybean yield.

Key words: Industrial seed treatment, biological nitrogen fixation, storage period, fungicide, insecticide, polymer, HiCoat, *Glycine max*.

INTRODUCTION

Inoculation of nitrogen-fixing bacteria of the *Bradyrhizobium* genus in soybean is a common practice in Brazil (Alves et al., 2003; Zilli et al., 2010). These bacteria are capable of fixing atmospheric nitrogen and provide it to the plant in exchange for photoassimilates (Bergersen, 1997; Fagan et al., 2007). As a result, the

*Corresponding author. E-mail: isandini@hotmail.com.

inoculation has allowed achievement of high soybean yields while reducing or even eliminating the need for application of nitrogen fertilizers (Mendes et al., 2003; Hungria et al., 2006; Machineski et al., 2018).

In order to ensure the viability of bacterial cells, farmers usually apply the inoculant on the soybean seeds at the day or up to 24 h before sowing (Silva Júnior et al., 2016). Application in-furrow during the sowing process can also be performed (Campo et al., 2010), but it is a less common practice due to the need for adaptation of planting machines and costs associated with it. The time required to inoculate the seeds on-farm has been considered a challenge from an operational point of view. In addition, seed treatment with insecticides and fungicides has often been performed to protect the seeds from pests, seed-borne and soil-borne pathogens (Goulart 1998), but the toxicity of these products has been considered a risk to people who manipulate the treated seeds as well as for the bacteria inoculated on the seeds with the purpose of fixing nitrogen. Therefore, new technologies need to be explored with the aim of overcoming these challenges.

As an alternative, seed companies have been adopting the industrial seed treatment, in which the company offers soybean seed already treated with insecticides, fungicides and pre-inoculated with *Bradyrhizobium* spp. (Brzezinski et al., 2015). This practice has allowed to speed up the operational process at sowing as well as to reduce the risk of exposure to toxic products. However, when fungicides and insecticides are added to the seed, the viability of the nitrogen-fixing bacteria could be jeopardized to a point where the number of viable bacteria is not enough to infect the plant effectively and form nodules (Hartley et al., 2012; Araujo et al., 2017). Thus, the compatibility of fungicides and insecticides represents a great challenge to the seed treatment (Campo et al., 2009; Pereira et al., 2010a; Silva Neto et al., 2013; Gomes et al., 2017). Very few studies were performed to evaluate the association of pre-inoculation and industrial seed treatment (Anghinoni et al., 2017; Machineski et al., 2018), and little is known about the maximum period of time before sowing that the seed could be treated without compromising the viability of the bacteria, nodulation of the plants and soybean yield (Pereira et al., 2010b; Zilli et al., 2010). Some authors have mentioned up to five days (Zilli et al., 2010), while other demonstrated that the period could be expanded to 30 days (Araujo et al., 2017) or even up to 60 days (Machineski et al., 2018) when a protectant is added to the inoculant.

Technologies that include polymers associated with the inoculation of *Bradyrhizobium* spp. could be an alternative to the conventional inoculants by avoiding the dehydration of the bacterial cells (Deaker et al., 2007) and protect them from toxic effects of fungicides and insecticides (Zilli et al. 2009; Campo et al. 2010). As a result, survival of the nitrogen-fixing bacteria could occur at levels that do not compromise the establishment of the symbiotic relationship and supply of nitrogen to the plant (Denardin and Freire, 2000; Fernandes Júnior et al., 2009; Pereira et al., 2010c; Fernandes Júnior, 2012; Marks et al., 2013; Araújo et al., 2017). Since little is known about the use of a new technology (HiCoat®) developed with the aim of enabling pre-inoculation of soybean seeds treated with fungicides and insecticides, the objectives of this study were i) to determine whether the HiCoat® technology allows the survival of *B. elkanii* after storage of the inoculated seeds; and ii) to evaluate whether the pre-inoculation and seed treatment with fungicides and insecticides reduces the physiological quality of seed and soybean yield.

MATERIALS AND METHODS

Seed treatments with fungicides and insecticides

All the fungicides and insecticides used in this study were commercial products. For application of the chemical treatments, 1 kg of seed of the soybean cultivar ND5909RR was placed into a plastic bag. For each treatment, the products were mixed according to their respective doses and water was added to bring the volume to 5 ml (Table 1). The mixture was applied on the seeds followed by agitation to uniformly distribute the products. The bags were maintained on a laboratory bench for the seeds to dry for 1 h. In all experiments, an untreated check without application of fungicide and insecticide was included.

Inoculant and inoculation procedure

The inoculants evaluated in this study were commercial products manufactured and commercialized by BASF S/A. Inoculant was applied using the HiCoat® technology, which is a mixture of 300 g of peat inoculant (Adhere HC®, *B. elkanii* strain SEMIA 587 and 5019 at 5×10^9 CFU ml^{-1}), 300 ml of liquid inoculant (Gelfix®, *B. elkanii* strain SEMIA 587 and 5019 at 5×10^9 CFU ml^{-1}), 150 ml of polymer S30 and 200 ml of distilled water. A volume of 9.5 ml kg^{-1} was applied on the seeds. For all experiments, an untreated check without inoculation was added along with a standard inoculation performed only with liquid inoculant (Gelfix®, *B. elkanii* strain SEMIA 587 and 5019 at 5×10^9 CFU ml^{-1}).

Survival of bacterial cells

Experiments were carried out under controlled conditions in the Laboratory of Field Crops of the Universidade Estadual do Centro-Oeste (UNICENTRO) in Guarapuava, Paraná, Brazil. The survival of *B. elkanii* was determined as the number of colony-forming units (CFU) recovered from soybean seeds according to the methodology established by Brazilian regulations (Mapa, 2010). A hundred seeds were placed into a 250-ml sterilized Erlenmeyer that contained 100 ml of 0.85% (w/v) NaCl solution with three drops of Tween 80. Flasks were agitated in an orbital shaker for 15 min. Serial dilutions in 0.85% (w/v) NaCl were prepared. A 100-µL aliquot from each dilution was placed onto sterilized Petri dishes containing CRYMA culture media (Fred and Waksman, 1928 cited by Mapa, 2010). After inoculation, the plates were maintained at 28°C for 10 days. The number of CFU was counted under an optical microscope, and only the plates between 30 to 300 colonies were considered. The results were expressed as number of CFU

Table 1. Seed treatments used in the experiments to evaluate the pre-inoculation of *Bradyrhizobium elkanii* on soybean seeds using the HiCoat® technology.

Treatment	Active ingrediente	Type of product[a]	Trade name	Dose of commercial product (ml kg^{-1})[b]	HiCoat[c]
1	Tiametoxan + Fludioxonil + Metalaxil-M + Abamectin	I + F + F + N	Cruiser® 600 FS + Maxim® Advanced + Avicta®	2 + 1 + 1	Yes
2	Fipronil + Fludioxonil + Metalaxil-M + Abamectin	I + F + F + N	Standak® + Maxim® XL + Avicta®	2 + 1 + 1	Yes
3	Fipronil + Thiophanate-methyl + Pyraclostroin + Abamectin	I + F + F + N	Standak® Top + Avicta®	2 + 1	Yes
4	Imidacloprid + Thiodicarbe + Fludioxonil + Metalaxyl-M + Abamectin	I + I + F + F + N	Cropstar® + Maxim® XL + Avicta®	2 + 1 + 1	Yes
5	Thiametoxan + Fludioxonil + Metalaxil-M + Abamectin	I + F + F + N	Cruiser® 350 FS + Maxim® XL	2 + 1	Yes
6	Fipronil + Fludioxonil + Metalaxil-M	I + F + F	Standak® + Maxim® XL	2 + 1	Yes
7	Fipronil + Thiophanate-methyl + Pyraclostroin	I + F + F	Standak® Top	2	Yes
8	Imidacloprid + Thiodicarbe + Fludioxonil + Metalaxyl-M	I + I + F + F	Cropstar® + Maxim® XL	2 + 1	Yes
9	-	-	-	-	Yes
10[d]	Fipronil + Thiophanate-methyl + Pyraclostroin	I + F + F	Standak® Top	2	No
11[e]	Fipronil + Thiophanate-methyl + Pyraclostroin	I + F + F	Standak® Top	2	No

[a]Type of product: I = insecticide; F = fungicide; N = nematicide. [b]All products were mixed and distilled water was used to bring the volume to 5 ml that was applied to one kilogram of seed. [c]HiCoat® Technology: a mixture of 300 g of peat inoculant (Adhere HC®, *Bradyrhizobium elkanii* strain SEMIA 587 and 5019 at 5 x 10^9 CFU ml^{-1}), 300 ml of liquid inoculant (Gelfix®, *Bradyrhizobium elkanii* strain SEMIA 587 and 5019 at 5 x 10^9 CFU ml^{-1}), 150 ml of polymer S30 and 200 ml of distilled water. [d]Treatment 10: standard inoculation was performed at the day of planting with liquid inoculant (Gelfix®. *Bradyrhizobium elkanii* strain SEMIA 587 and 5019 at 5 x 10^9 CFU ml^{-1}). [e]Treatment 11: no inoculation with *Bradyrhizobium elkanii* was performed.

per seed.

Effect of seed packing and storage period on survival of bacterial cells

The treatments consisted of two types of seed packing (plastic and paper), three seed treatments (Stadank® Top + standard inoculation; HiCoat® technology only; Standak® Top + HiCoat® technology), and seven storage periods (0, 3, 9, 15, 30, 45 and 60 days after inoculation). The bags used for seed packing were transparent plastic bags and brown paper bags. The dose of Standak® Top (fipronil + thiophanate-methyl + pyraclostrobin) was of 2 ml kg^{-1} of seed. The survival of *B. elkanii* was determined as described above. The study was carried out in a completely randomized design with three replications.

Effect of pre-inoculation on physiological quality of soybean seed

The experiment design was a complete randomized with eight replications. Eleven seed treatments were evaluated (Table 1). Fifty soybean seeds were placed on a "germitest" paper sheet previously soaked in distilled water, and another sheet was used to cover the seeds. The paper sheets were rolled and placed in an incubator at 25°C in the dark. Each sheet was considered as a replication. On the fifth day, seed vigor was evaluated according to Brasil (2009). The rolled papers were returned to the incubator for three more days and then germination, normal seedlings and non-germinated seeds were determined according to Brasil (2009). All data were expressed as percentage of the total seeds placed on each paper sheet.

Effect of pre-inoculation on nodulation of soybean plants

The experiment was carried out under field conditions in 1 m wide and 0.20 m height flowerbeds. The experimental design was a randomized complete block with four replications and eleven treatments (Table 1). Each experimental plot was composed of one row, and the rows were spaced 0.50 m apart. Twenty-five seeds were manually placed in each row at 2 cm deep and covered with soil. After emergence, only 10 seedlings were maintained in each row. At full flowering (R2) stage, three soybean plants were selected from each row. Plant root system was collected by placing a cylindrical metal device with 10 cm diameter and 10 cm height. The soil was washed off the roots and the nodules were collected with a metal screen. The number of nodules was determined. The nodules were placed in paper bags that were maintained in a drying oven at 65ºC for 72 h, and after weighting the dry mass was determined.

Effect of pre-inoculation on soybean yield

The experiments were conducted under field conditions in Guarapuava, Paraná, Brazil, during the 2010/2011 growing season. The experimental site is located in a region with approximately 1,100 m altitude and humid subtropical climate (Cfb). The soil was classified as a Brown Latosol (Embrapa, 2013). The experiment was carried out in a randomized complete block design, six replications and eleven treatments (Table 1). The experimental plot was composed of four rows spaced 0.40 m apart and 5.5 m length. The experiment was carried out twice. The first experiment was sowed on 28 Nov 2010 corresponding to the seed stored for 59 days, and the second experiment was sowed on 10 Dec 2010 with the seeds stored for 71 days.

Maize was grown during the previous summer season, while the experimental area remained under fallowing during the previous winter season. Desiccation was performed with glyphosate herbicide (720 g ha^{-1} a.i.) 30 days before sowing of soybean. Fertilization was performed at the sowing with 46 kg ha^{-1} of P_2O_5 and 60 kg ha^{-1} of K_2O. Management of weeds, pests and diseases was performed when needed.

Ten plants were collected from the two central rows of each plot. The number of pods and number of grains per plant were determined. The remaining plants in the two central rows of the experimental plot were manually harvested and threshed. The soybean seeds were weighted and seed moisture was determined. Soybean yield was determined in kg ha^{-1} at 13% moisture. A sample was taken and used to determine the thousand-grain mass by counting and weighing 300 grains.

Data analysis

Homogeneity of variances was analyzed by Bartlett's test. As a result, percentage values and counted values were transformed to$\sqrt{x+1}$. Analysis of variance was then performed, and means were compared by Tukey's Test at 5% of probability using the statistical program Sisvar (Ferreira, 2000).

RESULTS AND DISCUSSION

Survival of bacterial cells

The number of CFU recovered from soybean seeds was significantly affected by seed packing, seed treatment, storage period and all the interactions (Table 2). The survival of bacterial cells was reduced over time when soybean seeds received the standard seed treatment independently of seed packing, and at 60 days after inoculation no bacterial cells were recovered (Figure 1). However, when seeds were inoculated using the HiCoat® technology with and without the fungicide and insecticide, the number of CFU was maintained above 2 x 10^6 CFU per seed even at 60 days after inoculation (Figure 1).

Certain combinations of fungicides (Campo et al., 2009; Gomes et al., 2017; Costa et al., 2013) as well as insecticides (Pereira et al., 2010c) can be detrimental to *Bradyrhizobium* spp. due to the toxicity of the active ingredient, pH and solvents used in the formulations (Hungria and Campo, 2000), but the magnitude of the effect depends on the type and the period of contact between the chemicals and the inoculant (Costa et al., 2013). A previous study using a standard inoculation protocol with liquid products without polymers or any other type of protectant, demonstrated that 62% of bacteria inoculated on soybean seeds treated with fungicides died two hours after inoculation, and 95% of the bacteria died after 24 hours (Campo et al., 2009). In another study, the number of bacterial cells recovered from soybean seeds decreased in the first 2 h after inoculation, but it stabilized at 24, 48, 72 and 96 h after inoculation (Costa et al., 2013).

Nevertheless, in this study when the HiCoat® technology was used for inoculation, no significant adverse effect was observed on the survival of the bacterial cells. This technology includes a polymer, which may have acted as a protectant for the bacteria mainly against dehydration (Deaker et al., 2007) or toxicity of the chemical products (Pereira et al., 2010a). Previous observations confirmed the ability of polymers to protect cells from changes in the environmental conditions after inoculation of the seeds (Tittabutr et al. 2007). Use of polymers as carriers for inoculants allowed survival of *B. japonicum* for up to 180 days after inoculation of cowpea (Fernandes Júnior et al., 2009). Moreover, Araujo et al. (2017) pre-inoculated soybean seeds with commercial products that contained protectants, and verified that satisfactory populations of the *Bradyrhizobium* spp. were recovered from the seeds for up to 30 days after inoculation. All these results indicate that pre-inoculation can be used without compromising the survival of *Bradyrhizobium* spp. cells.

Table 2. Probability values from the analysis of variance for the effect of seed packing, seed treatment and storage period for the number of colony forming-units (CFU) of *Bradyrhizobium elkanii*.

Effect	Degrees of freedom	*P* values[a]
Seed Packing (SP)	1	<0.01
Seed Treatment (ST)	2	<0.01
Storage Period (S)	6	<0.01
SP x ST	2	<0.01
SP x S	6	<0.01
ST x S	12	<0.01
SP x ST x S	12	<0.01

[a]Variable was transformed to log_{10} for statistical analysis.

Table 3. Mean square and probability values from analysis of variance for the effects of seed treatment on the physiological quality of soybean seeds.

Effect	Degrees of freedom	Mean square values (*P* values)			
		Seed vigor[a]	Seed germination[a]	Abnormal seedlings[a]	Non-germinated seeds[a]
Seed treatment	10	0.6051 (0.002)	0.2356 (0.06)	0.5794 (0.31)	1.0185 (0.03)
Error	77	0.1908	0.1246	0.4849	0.4675
Mean		8.10	8.85	4.55	1.15
Coefficient of variation (%)		5.39	4.02	15.31	59.60

[a]Variables in percentage were transformed to √x+1 for statistical analysis.

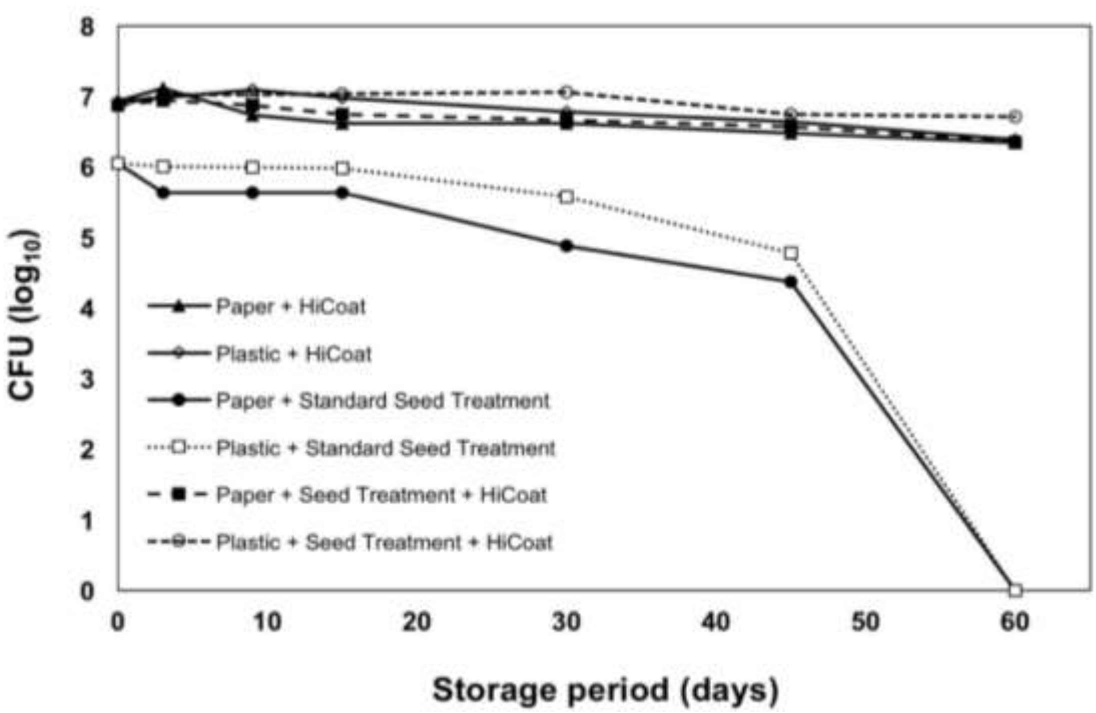

Figure 1. Mean colony forming-units (log_{10} of CFU per seed) of *Bradyrhizobum elkanii* for each seed packing (paper bag or plastic bag) and pre-inoculation with the HiCoat® technology or standard inoculation over different storage periods. Chemical seed treatment was performed with Standak® Top (fipronil + thiophanate-methyl + pyraclostrobin).

Table 4. Physiological quality of soybean seeds treated with different insecticides and fungicides and pre-inoculation at 71 days before sowing.

Treatment	Seed treatment	HiCoat®	Seed vigor (%)[a]	Seed germination (%)[a]	Abnormal seedlings (%)[a]	Non-germinated seeds (%)[a]
1	Tiametoxan + Fludioxonil + Metalaxil-M + Abamectin	Yes	67.50[ab]	77.50[a]	20.75[a]	1.75[ab]
2	Fipronil + Fludioxonil + Metalaxil-M + Abamectin	Yes	65.25[ab]	76.50[a]	23.00[a]	0.50[ab]
3	Fipronil + Thiophanate-methyl + Pyraclostroin + Abamectin	Yes	63.5 [ab]	73.25[a]	25.50[a]	1.25[ab]
4	Imidacloprid + Thiodicarbe + Fludioxonil + Metalaxyl-M + Abamectin	Yes	56.75[b]	75.00[a]	21.25[a]	3.75[a]
5	Thiametoxan + Fludioxonil + Metalaxil-M + Abamectin	Yes	63.25[ab]	77.00[a]	22.75[a]	0.25[ab]
6	Fipronil + Fludioxonil + Metalaxil-M	Yes	71.50[a]	82.25[a]	17.75[a]	0.00[b]
7	Fipronil + Thiophanate-methyl + Pyraclostroin	Yes	71.75[a]	82.25[a]	17.25[a]	0.50[ab]
8	Imidacloprid + Thiodicarbe + Fludioxonil + Metalaxyl-M	Yes	62.50[ab]	77.75[a]	21.50[a]	0.75[ab]
9	-	Yes	67.50[ab]	81.00[a]	17.75[a]	1.25[ab]
10[b]	Fipronil + Thiophanate-methyl + Pyraclostroin	No	67.50[ab]	79.75[a]	18.50[a]	1.75[ab]
11[c]	Fipronil + Thiophanate-methyl + Pyraclostroin	No	62.25[ab]	75.50[a]	21.50[a]	3.00[ab]

[a]Means with the same letter do not statistically differ at P ≤ 0.05 by Tukey's test. All statistical analysis and mean comparisons were performed on square-root-transformed data. [b]Treatment 10: standard inoculation was performed at the day of planting. [c]Treatment 11: no inoculation with *Bradyrhizobium elkanii* was performed.

Physiological quality of soybean seed

The seed treatments had significant effect on seed vigor and non-germinated seeds, but they did not affect the seed germination and abnormal seedlings (Table 3). Seed vigor reduced while the percentage of non-germinated seeds increased when imidacloprid + thiodicarbe + fludioxonil + metalaxyl-M + abamectin + HiCoat® were applied to the soybean seeds compared to fipronil + fludioxonil + metalaxyl-M + HiCoat®. However, when the treatment with imidacloprid + thiodicarbe + fludioxonil + metalaxyl-M + HiCoat® was applied, there was no significant difference from the other seed treatments (Table 4). Hence, the negative effect is due to the association of abamectin with imidacloprid + thiodicarbe + fludioxonil + metalaxyl-M rather than the active ingredients by themselves because no significant difference from other treatments was observed when abamectin was added with other combinations of fungicides and insecticides.

In this study, seed germination was not affected by the pre-inoculation of *B. elkanii*. In addition, none of the treatments with fungicides and insecticides associated with HiCoat® had lower seed germination when compared to the standard inoculation after 60 days of storage of the seeds. Similarly, neither peat-based inoculant nor liquid inoculant containing polymeric additives had an effect on the germination of soybean seeds (Tittabutr et al., 2007). It was previously showed that polymers can interfere with insecticide phytotoxicity, but did not affect the physiological quality of soybean seeds (Kumar et al., 2007; Pereira et al., 2010c; Camargo et al., 2017; Fagundes et al., 2017). The absence of a negative effect on the seed germination demonstrates that pre-inoculation can be performed on soybean seeds. However, caution must be taken to avoid the use of the imidacloprid + thiodicarbe + fludioxonil + metalaxyl-M + abamectin + HiCoat® due to the reduction of seed vigor. Low seed vigor can negatively affect the initial establishment of the plants and this can reduce soybean yield (Scheeren et al., 2010).

Nodulation of plants

Biological nitrogen fixation by *Bradyrhizobium* spp. is complex and the efficiency of the nodulation is mediated by internal factors, for example plant hormones and

Table 5. Mean square and probability values from analysis of variance for the effects of seed treatment on the nodulation of soybean plants.

Effect	Degrees of Freedom	Mean square values (*P* values)		
		Number of nodules per plant[a]	Mass of nodules per plant (g)[a]	Mass of nodule (mg)
Block	3	2.7695 (0.006)	0.01445 (0.09)	1.2983 (0.15)
Seed Treatment	10	0.9620 (0.11)	0.0076 (0.33)	1.0756 (0.16)
Error	30	0.5427	0.0064	0.6781
Mean		8.71	0.3653	4.89
Coefficient of Variation (%)		8.46	21.84	16.85

[a]Variables in percentage were transformed to $\sqrt{x+1}$ for statistical analysis.

availability of photoassimilates, along with external factors such as temperature, oxygen, water, nutrients and products applied during seed treatment (Fagan et al., 2007). In this study, there was no significant effect of pre-inoculation associated with fungicides and insecticides on the number of nodules per plant, mass of nodules per plant and mass per nodule (Table 5). Similarly, in a study performed by Pereira et al. (2010a), the treatment of soybean seeds with the fungicides carbendazin + thiram or thiabendazole + thiram associated or not with polymer, and independently of the application time, did not affect the establishment and development of nodules when the seeds were inoculated with *Bradyrhizobium*. Moreover, there was no significant difference among the pre-inoculation of cowpea seeds with either polymer or peat inoculant until 14 days after inoculation, but at 35 days the nodulation and dry mass with polymer inoculant was significant higher than the peat inoculant (Silva Júnior et al., 2016).

The non-inoculated control (Table 6) had the lowest number and mass of nodules per plant, but the difference from the other treatments was not significant due to the high variation of the data. The presence of nodules on the plants for the non-inoculated control (Table 6) was due to natural contamination by nitrogen-fixing bacteria as observed in the study by Pereira et al. (2010a). Naturalized population of *Bradyrhizobium* spp. in the soil of a field previously grown with soybean can provide satisfactory levels of nodulation and no response by the application of different doses of inoculant may be observed (Campos 1999). However, Nishi and Hungria (1996) verified that even in soils with elevated population of *Bradyrhizobium* spp. there was increase in the number and mass of nodules by the inoculation.

Soybean yield

Soybean yield and thousand grain mass were significantly affected by experiment (Table 7). The means of these two variables were significantly lower in the experiment 2 compared to Experiment 1 (Table 8). The difference in yield between the two experiments was likely due to the planting time since soybean is sensitive to photoperiod (Farias et al., 2007) rather than the difference in the storage period of the seeds because there was significant lower yield in the standard inoculation treatment (Table 8). At the latitude in the Southern Hemisphere that this study was performed, the photoperiod in December is longer than November due to the approach of summer solstice. Thus, sowing carried out in November leads to development of taller plants that are also more productive when compared to plantings in December, in which earlier flowering occurs even when foliar index is still low (Fietz and Rangel, 2008).

There was no significant effect of pre-inoculation and seed treatment with fungicides and insecticides as well as the interaction between seed treatment and experiment on soybean yield, thousand grain mass, number of pods per plant and number of grains per pod (Table 7). Similarly, Machineski et al. (2018) verified that pre-inoculation of soybean seeds using a cell protector was efficient in maintaining the bacterial inoculant viable on the seed for up to 60 days and did not negatively affect soybean yield. Anghinoni et al. (2017) also verified that soybean seeds industrially treated with fludioxonil fungicide and thiametoxan insecticide can be inoculated and stored up to 10 days before sowing, with no adverse effects on grain yield. In addition, Silva Júnior et al. (2016) found out that cowpea yield was not significantly different from the standard inoculation and positive control (70 kg ha^{-1} of N) when peat and polymer inoculants were used (Silva Júnior et al., 2016). All these results are contrary to what was found by Brzezinski et al. (2015) and Zilli et al. (2009), where the authors mentioned that treatment of soybean seeds with insecticide and fungicides before sowing hinders the establishment of soybean in the field, and reduces nodulation of the plants, respectively. However, the difference may be due to the absence of polymers in both studies and the fact that seeds were stored for 240 days before sowing in the work by Brzezinski et al. (2015).

This study was performed in a Brown Latosol, with

Table 6. Nodulation of soybean plants with pre-inoculation of *Bradyrhizobium* using the HiCoat® technology and different fungicide and insecticide seed treatments.

Treatment	Seed treatment	HiCoat®	Nodulesperplant[a]	Massofnodulesperplant(g)[a]	Massofnodule(mg)[a]
1	Tiametoxan + Fludioxonil + Metalaxil-M + Abamectin	Yes	70.50[a]	0.36[a]	5.16[a]
2	Fipronil + Fludioxonil + Metalaxil-M + Abamectin	Yes	88.08[a]	0.38[a]	4.36[a]
3	Fipronil + Thiophanate-methyl + Pyraclostroin + Abamectin	Yes	78.38[a]	0.41[a]	5.17[a]
4	Imidacloprid + Thiodicarbe + Fludioxonil + Metalaxyl-M + Abamectin	Yes	70.50a	0.40[a]	5.67[a]
5	Thiametoxan + Fludioxonil + Metalaxil-M + Abamectin	Yes	71.10[a]	0.40[a]	5.56[a]
6	Fipronil + Fludioxonil + Metalaxil-M	Yes	71.42[a]	0.34[a]	4.79[a]
7	Fipronil + Thiophanate-methyl + Pyraclostroin	Yes	84.08[a]	0.37[a]	4.44[a]
8	Imidacloprid + Thiodicarbe + Fludioxonil + Metalaxyl-M	Yes	72.50[a]	0.37[a]	5.25[a]
9	-	Yes	77.17[a]	0.36[a]	4.85[a]
10[b]	Fipronil + Thiophanate-methyl + Pyraclostroin	No	87.83[a]	0.39[a]	4.42[a]
11[c]	Fipronil + Thiophanate-methyl + Pyraclostroin	No	60.08a	0.25[a]	4.10a

[a]Means followed by the same letter do not statistically differ by Tukey's Test at $P \leq 0.05$. [b]Treatment 10: standard inoculation was performed at the day of planting. [c]Treatment 11: no inoculation with *Bradyrhizobium elkanii* was performed.

Table 7. Mean square and probability values from analysis of variance of the effects of seed treatment on the soybean yield and yield components.

Effect	Degress of fredom	Mean square (*P* values)			
		Yield (kg ha^{-1})	Thousand grain mass (g)	Number of grains per plant	Number of pods per plant
Block	5	108547 (0.01)	15.25 (0.34)	171.38 (0.41)	30.72 (0.37)
Experiment (E)	1	12674135 (<0.01)	789.09 (<0.01)	378.46 (0.14)	62.49 (0.14)
Seed Treatment (ST)	10	17185 (0.90)	14.33 (0.38)	49.83 (0.98)	9.50 (0.97)
E x ST	10	12900 (0.96)	9.07 (0.73)	38.85 (0.99)	6.32 (0.99)
Error	105	36081	13.18	169.01	28.04
Mean		3487	153.52	95.66	46.72
Coefficient of Variation (%)		5.45	2.36	13.59	11.33

elevated content of clay and high organic matter. In a study performed under field conditions with sandy soil with low organic matter content and with soybean grown for the first time, Zilli et al. (2010) did not observe significant difference between the pre-inoculation at 5 days before planting compared to the standard inoculation on the number and mass of nodule formed on soybean plants, and both were superior to the check and nitrogen fertilization. Therefore, studies need to be carried out under different field conditions to effectively determine the effect of pre-inoculation of soybean seeds.

Scientific, technological and commercial challenges need

Table 8. Yield and yield components of soybean with pre-inoculation of *Bradyrhizobium elkanii* using the HiCoat® technology and different fungicide and insecticide seed treatments in two experiments under field conditions.

Treatment	Seed treatment	HiCoat®	Yield (kgha^{-1})^{a}		Thousand grain mass (g)^{a}		Number of grains per plant^{a}		Number of pods per plant^{a}	
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2	Exp1	Exp 2
1	Tiametoxan + Fludioxonil + Metalaxil-M + Abamectin	Yes	3886^{aA}	3161^{aB}	158.22^{aA}	151.22^{aB}	96.99^{aA}	95.89^{aA}	46.62a^{A}	48.75^{aA}
2	Fipronil + Fludioxonil + Metalaxil-M + Abamectin	Yes	3782^{aA}	3165^{aB}	154.67^{aA}	151.88^{aA}	95.42^{aA}	94.30aA	45.68^{aA}	46.60^{aA}
3	Fipronil + Thiophanate-methyl + Pyraclostroin + Abamectin	Yes	3763^{aA}	3187^{aB}	154.73^{aA}	151.88^{aA}	97.43^{aA}	97.19^{aA}	45.57^{aA}	47. ^{aA}
4	Imidaclopridо + Tiodicarbe + Fludioxonil + Metalaxyl-M + Abamectin	Yes	3795^{aA}	3292^{aB}	154.42^{aA}	150.47^{aA}	98.71^{aA}	92.35^{aA}	47.17^{aA}	46.40^{aA}
5	Tiametoxan + Fludioxonil + Metalaxil-M + Abamectin	Yes	3784a^{A}	3099^{aB}	156.27^{aA}	149.48^{aB}	98.01^{aA}	89.73^{aA}	44.80^{aA}	46.57^{aA}
6	Fipronil + Fludioxonil + Metalaxil-M	Yes	3829^{aA}	3220^{aB}	158.54^{aA}	152.27^{aB}	99.49^{aA}	94.12^{aA}	47.62aA	48.57^{aA}
7	Fipronil + Thiophanate-methyl + Pyraclostroin	Yes	3777^{aA}	3208^{aB}	155.50^{aA}	149.96^{aB}	98.58^{aA}	93.31^{aA}	45.54aA	48.22^{aA}
8	Imidaclopridо + Tiodicarbe + Fludioxonil + Metalaxyl-M	Yes	3844^{aA}	3140^{aB}	155.27^{aA}	152.82^{aA}	91.55^{aA}	94.13^{aA}	44.08aA	48.13^{aA}
9	-	Yes	3809^{aA}	3175^{aB}	158.39^{aA}	151.49^{aB}	103.97^{aA}	96.06^{aA}	46.72^{aA}	47.05^{aA}
10[b]	Fipronil + Thiophanate-methyl + Pyraclostroin	No	3793^{aA}	3176^{aB}	155.34^{aA}	150.41^{aB}	95.34^{aA}	95.44a^{A}	48.20^{aA}	47.45^{aA}
11[c]	Fipronil + Thiophanate-methyl + Pyraclostroin	No	3699^{aA}	3122^{aB}	154.29^{aA}	149.98^{aB}	95.35^{aA}	91.09^{aA}	44.28^{aA}	46.38^{aA}
Mean			3796 ^{A}	3177 ^{B}	155.97^{aA}	151.08^{B}	97.35^{A}	93.96^{A}	46.03^{A}	47.4^{aA}

[a]Means followed by the same capital letter for experiments and lower case for seed treatment do not statistically differ by Tukey's test at P ≤ 0.05. [b]Treatment 10: standard inoculation was performed at the day of planting. [c]Treatment 11: no inoculation with *Bradyrhizobium elkanii* was performed.

to be overcome to allow the use of new seed treatments at a global scale (O'Callaghan, 2016). Some authors have suggested that if pre-inoculation of soybean seeds is desired, some protective inoculant technology must be used (Araujo et al. 2017). Our findings indicated that the fungicide and insecticide associated with the HiCoat® technology can be used in the industrial seed treatment and seeds can stored for up to 71 days without compromising the nodulation of plants and soybean yield. Further studies are required to investigate the effect of pre-inoculation using the HiCoat® technology with other species and strains of *Bradryrhizobium*, different soybean cultivars, and conditions of sandy soil with low organic matter content and no history of cultivation of soybean.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Comparative assessment of the fumigant action of volatile oils from three garlic cultivars on faba bean beetle *Bruchidius incarnatus* (Boh.)

Azhari Omer Abdelbagi, Mubarak Youssof El-sonoussy Eissa, Abd Elaziz Sulieman Ahmed Ishag* and Ahmed Mohammed Ali Hammad

Department of Crop Protection, Faculty of Agriculture, University of Khartoum, Sudan.

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The study aims to assess the insecticidal activity, oviposition inhibitory and ovicidal effects of volatile oils from different garlic cultivars on faba bean beetle, *Bruchidius incarnatus* (Boh.) (Chrysomelidae: Bruchinae). The oils were tested at various concentrations and time intervals. Results showed that all volatile oils caused significant mortality to the test insects. The effects were time and dose-related. The 72 h median lethal doses ($LD_{50\%}$) of male and female *B. incarnatus* were 0.03 and 0.006% (Chinese oil); 3.77 and 0.06% (Egyptian oil) and 0.03 and 3.77% (Sudanese). The respective median lethal times ($LT_{50,}$ at 0.01%) for male and female were 55.88 and 57.57 h (Chinese oil); 48.62 and 57.76 h (Egyptian oil) and 79.93 and 84.99 h (Sudanese oil). The results of egg hatchability of *B. incarnatus* exposed to the lowest concentration (0.01%) were: 31, 32 and 34% for Chinese, Egyptian and Sudanese oils, respectively. The respective median lethal doses (LD_{50} %) for Chinese, Egyptian and Sudanese oils were 0.025, 0.034 and 0.21%. The results indicated that there were significant differences among the three garlic oils; however, the efficacy of the three types of garlic oils against the test insects can be in this order: Chinese> Egyptian> Sudanese oil.

Key words: Bruchinae, fumigation, faba bean, garlic.

INTRODUCTION

In Sudan, faba bean constitutes an important component of human diet both in rural and urban areas. The crop is grown in 58,000 ha in Sudan, with an average yield of about 251 kg/ha (FAO, 2001). Sixty percent of the cultivated land is allotted to faba bean in Northern State, and 3.6% in the Nile State. Most of the production comes from the Northern and River Nile states, with a small amount from Khartoum, Gezira, Jebal Marra and Rahad Schemes (Abdul-Bari and Nygaard, 1982). The post-harvest losses have been estimated at 5 to 10% with the majority attributed to insect infestation. The duration of storage affects the relative abundance and succession of insects. The major storage pests of faba bean in the Sudan are the faba bean beetle *Bruchidius incarnatus*

*Corresponding author. E-mail: a.aziz30@gmail.com. Tel: +249915378530.

(Boh.) (Chrysomelidae: Bruchinae). Infestations by these insects lead to serious losses in quantity, quality, and germination percentage of stored faba bean. Insect damage to stored grains and pulses may reach up to 40% in countries where modern storage technologies have not been introduced. Currently, the measures to control pest infestation in grain and dry food products rely heavily upon the use of gaseous and liquid insecticides, which may pose possible health hazards to warm-blooded animals and an increased concern to the environment. Fumigation is still one of the most effective methods used for the protection of stored food, feeds stuff and other agricultural commodities from insect infestation. However, the number of chemicals for insect control decreased drastically as problems of insect resistance intensified and social pressures against the use of toxic chemicals in stored food started to gain increased concern for health environmental reasons (Varma and Dubey, 1999). Based on this the requirements for safer means of pest management became crucial. Therefore, the use of safe, low toxic botanical pesticides is now emerging as one of the prime means to protect crops and their products as well as the environment from pollution by pesticides (Prakash and Rao, 1997).

Botanical pesticides can cause less damage to human health and environment than conventional insecticides. Many of them degrade rapidly and do not accumulate in the body and environment while some are very pest specific and do little or no damage to other organisms. The body of scientific literature documenting bioactivity of plant derivatives to arthropod pests continues to expand, yet only a handful of botanicals are currently used in agriculture in the industrialized world, and there are few prospects for commercial development of new botanical products (Nabil, 2013). Garlic products are marketed in the European Union (EU) (Commission Directive, 2003) as foodstuffs as well as herbal medicinal products. Majority of these products are sold with health claims. In contrast, garlic medicinal products are available only in a limited number of EU member states. This difference is due to discrepancies among the national laws of EU member states. European legislation does not distinguish between medicinal products prepared from chemical substances and those produced from plants or other natural substances. However, garlic is regulated by the same rules as all other medicinal products. In general, manufacturers of herbal products have difficulty in satisfying all the requirements of European directives and guidelines for medicinal products (Commission Directive, 2003; Krose, 2006).

The insecticidal activity of a large number of essential oils and other plant extracts has been assessed against several major agricultural pests (Regnault-Roger et al., 1993; Park et al., 2003; Park et al., 2006; Ahmed and Abdelbagi, 2014; Sir El Khatim and Abdelbagi, 2015; Abdalla et al., 2017; Sir El Khatim et al., 2018). One of the promising sources of natural products is garlic *Allium sativum* (Stoll, 2001). Garlic volatile oil has insecticidal, repellent, antifeedant, antibacterial, nematicidal, and acaricidal properties (Tandon and Lal, 1980). The relative toxicity of garlic oil to adult and larvae of house fly *Musca domestica* Fabr. and *Trogoderma granarium* has been reported (Bhatnagar and Pal, 1974). Xin et al. (2014) and Douiri et al. (2013) identified the chemical composition of the garlic essential oil as dimethyl disulfide, diallyl sulfide, allyl isothiocyanate, allyl methyl disulfide, dimethyl trisulfide, 1,3-dithiane, diallyl disulfide, linalool, allyl methyl trisulfide, borneol, methyl propyl trisulfide, α-Terpineol, dimethyl tetrasulfide, diallyl trisulfide, diallyl thiosulfinate, allyl methyl tetrasulfide and diallyl tetrasulfide. The principal compound found in the essential oil was diallyl trisulfide (61.87%) followed by diallyl disulfide (19.47%), diallyl sulfide (4.41%) and diallyl tetrasulfide (3.67%). Xin et al. (2014) observed that the two main constituents of *A. sativum* oil (diallyl trisulfide and diallyl disulfide) exhibited strong fumigant activity against the whitefly, *Bemisia tabaci* adults. Diallyl trisulfide shows stronger fumigant activity than the crude essential oil and diallyl disulfide. Moreover, diallyl trisulfide exhibited 20 times stronger fumigant activity against the whitefly than other constituent compound. Further, they also suggested that the fumigant activity of the essential oil of *A. sativum* may be attributed to diallyl trisulfide (Xin et al., 2014).

In Sudan, Ahmed (1998) reported the promising potential of the fumigant action of garlic oil in the control of stored products pests. Abdalla (2003) evaluated the fumigant action of garlic volatile oils against major storage pests and revealed the insecticidal properties of local garlic cultivar volatile oil with promising results. Khiralla (2007) evaluated the efficacy of oil extract from Chinese garlic cultivar against two major bruchids. Taha (2007) reported the efficacy of Egyptian garlic cultivar oil against two stored legumes pests. The efficacy of garlic oil and aqueous extract against the Cowpea beetle was reported (Ahmed and Abdelbagi, 2014; Abdalla et al., 2017). Based on the promising results obtained by previous authors this present study was conducted to investigate the use of volatile oils from three garlic cultivars (Chinese, Egyptian and Sudanese) as fumigants against the adult of broad bean beetle *B. incarnatus* (Boh.), and to study the insecticidal efficacy, antioviposition and ovicidal effects of these oils.

MATERIALS AND METHODS

Insect culture

The source of faba bean infested with *B. incarnatus* (Boh.) was Dongola Research Station, Agricultural Research Corporation, Sudan. The obtained culture was brought to the Department of Crop Protection, Faculty of Agriculture Laboratory and sieved by mesh No. 10 to remove adult insects. Adults were reared in rearing glass jars of 3 kg, and half filled with sound grain of faba bean local variety; each jar was covered with a muslin cloth, fixed with rubber bands and kept in the laboratory at room temperature. Newly emerged (24 h) insects were used. Females were kept for 10-15 min with a surplus of newly emerged males, to allow mating (Ahmed, 1998).

Preparation of garlic (*A. sativum* L.) volatile oils

The volatile oils of the three cultivars of garlic (Chinese, Egyptian and Sudanese) were tested against adult and eggs of faba bean beetle *B. incarnatus*. Volatile oils of Chinese and Sudanese cultivars were obtained by the extraction procedure described below, while oil of Egyptian cultivar was obtained as ready-made oil from an Egyptian company (El-captain Company for Extraction of Natural oils, CAP PHARM, Cairo, Egypt). The mortality, fecundity, and ovicidal effects were assessed following exposure to the fumes emanating from garlic volatile oils (Abdalla, 2003; Khiralla, 2007; Taha, 2007).

Garlic preparation

The cloves of the garlic were cleaned, sliced and dried under shade in dark room for 19 days in winter and 11 days in summer. The dried garlic was ground using kitchen grinder “Moulinex”. The powder was subjected to steam distillation and soxhlet extraction using ethanol (Guenther, 1961).

Garlic oil preparation

Steam distillation

Steam distillation was done according to the method of Abdalla (2003). 300 g of Chinese garlic powder was placed in five liters round bottom flask and 1.5 L of distilled water was added. The mixture was thoroughly shaken for 20 min manually. The content was subjected to steam distillation at 65 to 70°C for 3 h. Anhydrous sodium sulfate (0.1 g/mL oil) was added to absorb the moisture. Oil was collected in glass containers and kept in a refrigerator at 4°C for bioassay (Abdalla and Abdelbagi, 2015).

Ethanol extract

Sudanese garlic powder (500 g) was placed in five liters round bottom flask and 2 L of ethanol (96%) was added. The mixture was subjected to Soxhlet extraction at 60 to 70°C for 6 h. The oil obtained was kept in a refrigerator at 4°C for bioassay.

Fumigation chamber

Airtight glass (5 mm thickness) fumigation chambers (16×16×16 cm) with sliding doors were prepared according to Abdallah (2003). Small pieces of glass (4×4 cm) were sealed together using Silicon adhesive. A stool of wire net was laid open inside the fumigation chamber to hold the jute sacs during the fumigation process.

Sexing of B. incarnatus

Sexing was done based on the morphology of antennae, pygidia and abdominal sternite as described by Shomar (1963). Sexing was also done based on Elkifl and Metwally (1971) who stated that the pygidia of the male and the female are characteristically different; those of the female have two large dark colored patches on the dorsal side separated by a line of white pubescence; in male such patches are lacking and the male last abdominal sternite is not emarginated.

Separation of eggs

The eggs of *B. incarnatus* were isolated according to Ahmed (1987) who stated that the eggs are small (less than 1 mm in length), smooth, oval in shape, flattened at the dorsal and ventral sides and rather transparent in color when newly laid. The eggs glued to the seed tests and were laid singly.

Toxicity assay

The mortal effect of garlic volatile oils on the adults of *B. incarnatus* was assessed. Twenty adults (10 males and 10 females) of 24 h old and of the same size were chosen from the main culture. To obtain the adults, the culture medium was sieved to remove the adult beetles therein and the adults emerging in the following day were collected for the experiment. The test insects were introduced into small jute sacs containing 50 g of sound grains of local faba bean and/or cowpea varieties purchased from the local market; they were recently harvested, free from any infestation and never received fumigation treatments. Sacs were then laid over the wire net stools and placed into a different fumigation chamber. Series of dilutions (10, 5, 1, 0.1, and 0.01%) of each type of garlic oils were prepared using ethanol 96% as a solvent, and diluted to 10% with distilled water. Petri dishes (covered with muslin cloth fixed with a rubber band to avoid insects from falling in the fumigant) containing 2.5 mL of each concentration were placed beneath the jute sack. The amount of each oil ranged from 0.5 to 5 ml/kg. The mortality of the test insects was calculated every 24 h for a week. The test insects without legs, antennal movement and not reacting to probing were considered dead. Recoveries of test insects were observed for a week. The experiment was kept in an incubator (Gallenkamp) at 28 to 35°C and 60 to 70% relative humidity (Khiralla 2007).

The experimental units were arranged in a completely randomized design with four replicates. Two controls treated with only the solvent or distilled water were included.

Oviposition inhibitory test

The oviposition inhibitory property of garlic volatile oils was determined by fumigating newly emerged adults. Different concentrations of garlic volatile oils were used. Ten pairs of *B. incarnatus* (10 males and 10 females) of 24 h old and of the same size were chosen from the main culture. To obtain the 24 h old adults, the culture medium was sieved to remove the adult beetles therein and the adults emerging in the following day were collected for the experiment. The test insects were introduced into small jute sacs containing 50 g of sound grains of faba bean and/or cowpea local varieties purchased from the local market. Sacs were placed over wire net stools and the fumigation chamber. Series of dilutions (10, 5, 1, 0.1, and 0.01%) of each type of garlic volatile oils were prepared using ethanol (96%) as a solvent, diluted to 10% with distilled water. Petri dishes (covered with muslin cloth fixed with a rubber band to avoid insects from falling in the fumigant) containing 2.5 ml of each concentration were placed beneath the jute sacs. Mating took place soon after emergence of the adults indicating that both sexes have fully developed sex organs. The number of eggs laid was counted every 24 h for a week (Abd El-Aziz, 2001). The temperature and RH were 28 to 35°C and 60 to 70%, respectively.

The experimental units were arranged in a completely randomized design with four replicates. Two controls treated with only the solvent or distilled water were included.

Ovicidal assay

An assay was carried out to study the ovicidal property of Chinese, Egyptian and Sudanese garlic volatile oils. Ovicidal test of the volatile oils was done by fumigating eggs with different concentrations. Adults of tested insects *of* 24 h old were allowed to lay their eggs on sound grains of local faba bean and/or cowpea varieties for 24 to 72 h. Seeds bearing 1 to 5 eggs were selected from the culture using a hand lens. A total of fifty eggs were tested per replicate, and selected seeds with their eggs were introduced into opened plastic cups (Ibrahim, 2000).

Series of dilutions (20, 10, 1, 2, 0.1, 0.05 and 0.01%) (V/V) of each cultivar of garlic oils were prepared using ethanol (96%) diluted to 10% using distilled water. 5 ml of each concentration was placed in a glass Petri-dish and in the fumigation chambers. The exposure period was a week. The fumigated eggs were transferred to glass jars of 250 g; they were covered with muslin cloth, fixed with rubber bands and kept in an incubator (Gallenkamp) at 32°C and 60 to 70% RH. The emerged insects from the test eggs were counted every 24 h for 17 days. The eggs were considered dead if not hatched after 5 days before the end of the experiment (that is after 22 days). The experiment was kept at 28 to 35°C and 60 to 70% RH (Sabbour and Abd-El-Aziz, 2010).

The experimental units were arranged in a completely randomized design with four replicates. Two controls with only the solvent or distilled water were included.

Statistical analysis

The data collected were expressed as percentage and subjected to the analysis of variance based on the procedure described by Gomez and Gomez (1984), using SAS software for Windows version (2004). The median lethal doses (LD_{50}) and median lethal times (LT_{50}) were calculated following the probit analysis method of Finney (1971) using Minitab software version 13.3 (Minitab, 2000).

RESULTS AND DISCUSSION

Insecticidal effect of garlic volatile oils on *B. incarnatus* (adults)

The analysis of the data showed that all three cultivars of garlic volatile oils induced greater mortality of test insects (male and female) compared to the control.

Toxicity assay

The results in Figures 1 to 6 indicated that all exposure periods (24 to 168 h) to garlic volatile oils caused significant mortality to the test insect (*B. incarnatus*) compared to the control. The Chinese garlic volatile oil was the most effective with initial rapid action causing 100% mortality against both sexes at the concentration of 5% or greater within 24 h; its two lowest concentrations (0.1 and 0.01%) reached at least over 80% in both sexes within 96 h (Figures 1 and 4). On the other hand, the Egyptian garlic volatile oil ranked second with initial rapid action that causes complete mortality of both sexes at the highest and medium concentrations (1, 5, and 10%) within 72 h; however, its two lower concentrations perform slightly better than the Chinese oil causing over 75% mortality in both sexes within 72 h (Figure 3). However, the Sudanese garlic volatile oil appears to have the slowest and least effective action; its two highest concentration caused over 80% mortality after 96 h, while its two lower concentrations (0.1 and 01%) caused over 75% mortality in both sexes after 144 h (Figures 4 to 5). Results showed that 24 h exposure had the highest mortality. However, the highest mortality with Egyptian cultivar was 50% against both sexes at 10%. While Sudanese cultivar induced 52.5% mean mortality against male and 47.5% against the female at 10% (Figure 1). Responses of both sexes were dose-related. In the male, the top four concentrations of the three oil types are significantly different from the control and the female response followed similar trend except in case of Chinese cultivar oil where all concentrations are significantly different from the control set.

However, the result of 48 h shows that the highest mortality was recorded with Chinese garlic volatile oil which caused 100% mortality in both sexes at 5 and 10%. The highest mortality with Egyptian cultivar was 77.5% in male at 10%. While Sudanese cultivar induced 60% mortality in male and 55% in female at 10% (Figure 2). On the other hand, the results of 72 h showed that the highest mortality was recorded with Chinese garlic volatile oil which caused 100% mortality in male at 1, 5 and 10% concentrations and in female at concentrations of 5 and 10%. The highest mortality caused by the Egyptian cultivar was 100% in male at concentrations of 5 and 10%. While Sudanese cultivar induced 82.5% mortality in both sex at a concentration of 10% (Figure 3).

The results of 96 h showed that the highest mortality was recorded with Chinese garlic volatile oil which caused 100% mortality in male at the concentration of 1, 5, and 10% and in female at concentrations of 5 and 10%. The Egyptian cultivar caused 100% mortality at all concentrations in both male and female. While Sudanese cultivar recorded 87.5% mortality in both sexes at concentration of 10% (Figure 4). Both Chinese and Egyptian garlic volatile oils caused 100% mortality in both male and female of the test insect at all concentrations after 144 h. While the Sudanese cultivar induced 100% mortality in both male and female at concentrations of 5 and10% (Figure 5). After 168 h of exposure, the statistical analysis of the data showed that the highest mortality was recorded with Chinese and Egyptian oils which caused 100% mortality in male and female at all concentrations. While Sudanese type induced 100% mortality in male at a concentrations of 0.01 and 100% female mortality at concentrations of 5 and 10% (Figure 6).

All treatments were significantly different from the control sets (Figures 1 to 6) and responses of both sexes

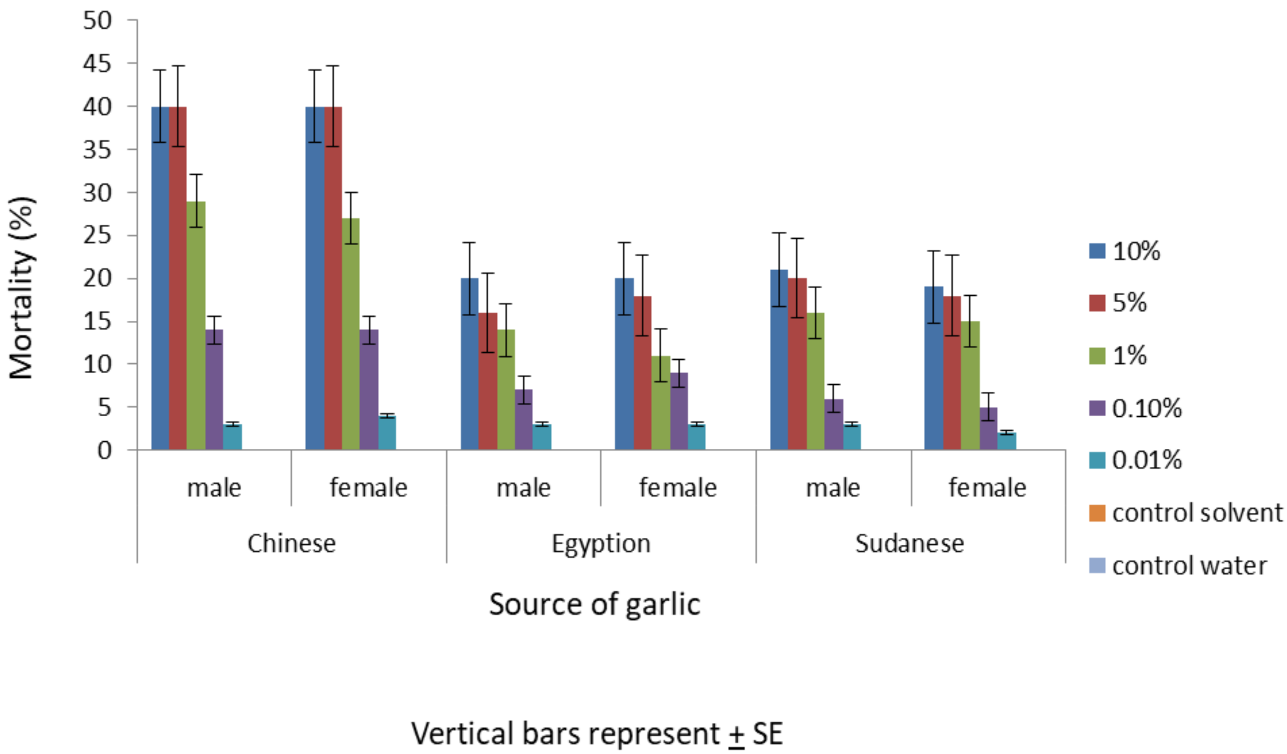

Figure 1. The 24 h mortality of male and female of faba bean beetle *Bruchidius incarnatus* exposed to garlic volatile oils.

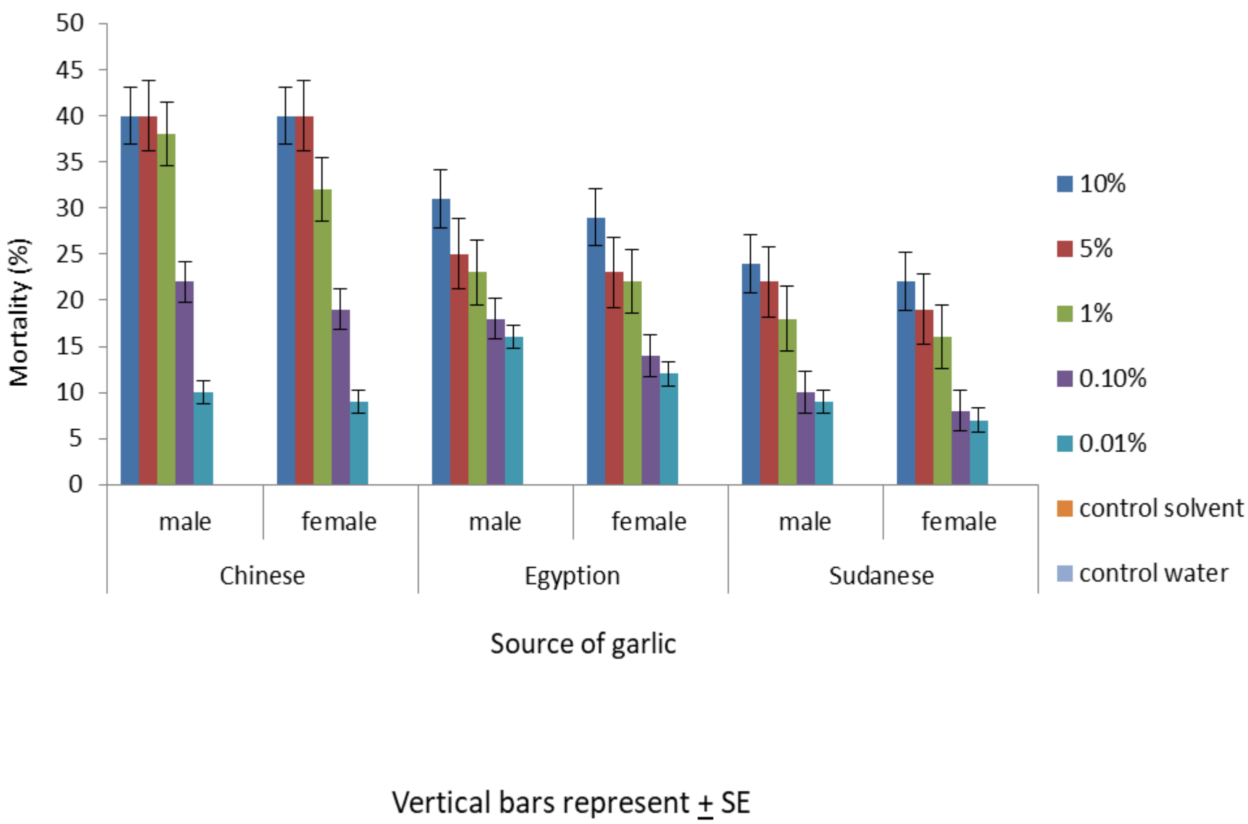

Figure 2. The 48 h mortality of male and female *Bruchidius incarnatus* exposed to garlic volatile oils.

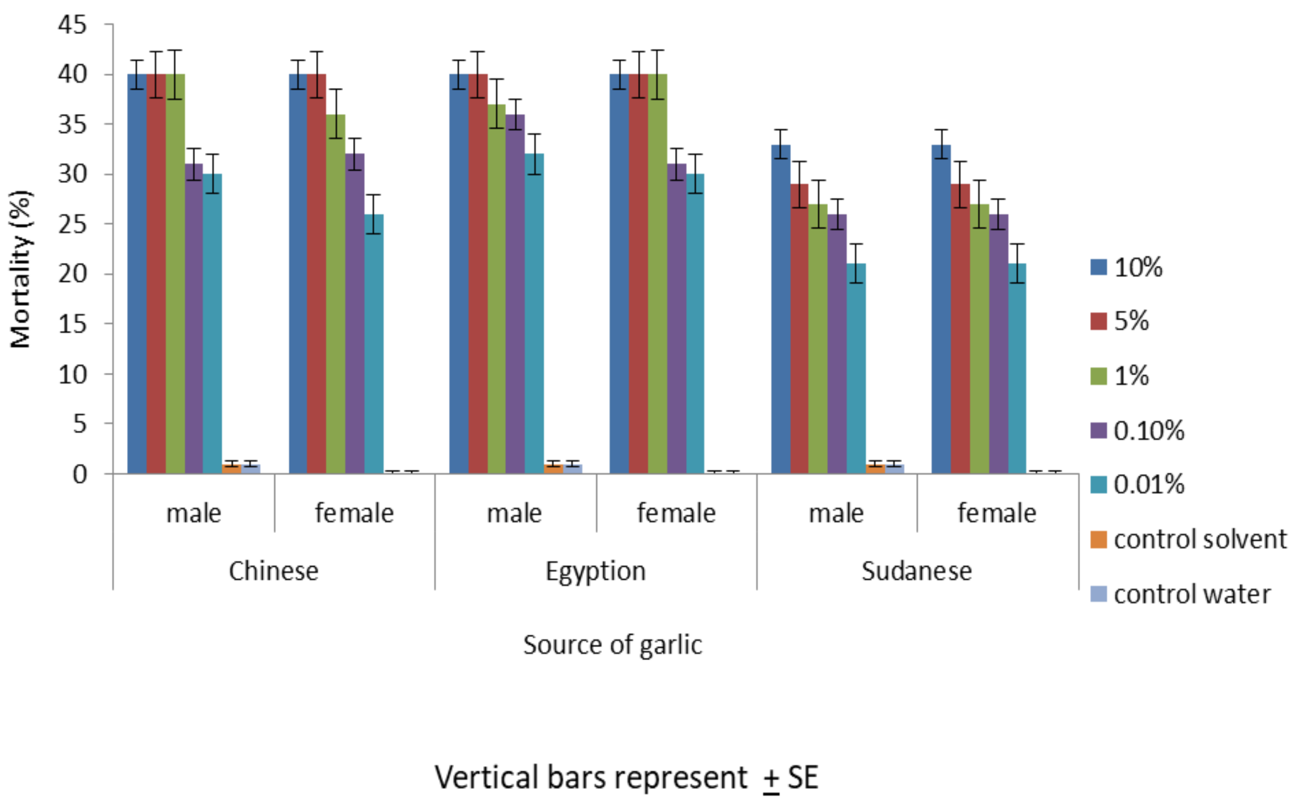

Figure 3. The 72 h mortality of male and female *Bruchidius incarnatus* exposed to garlic volatile oils.

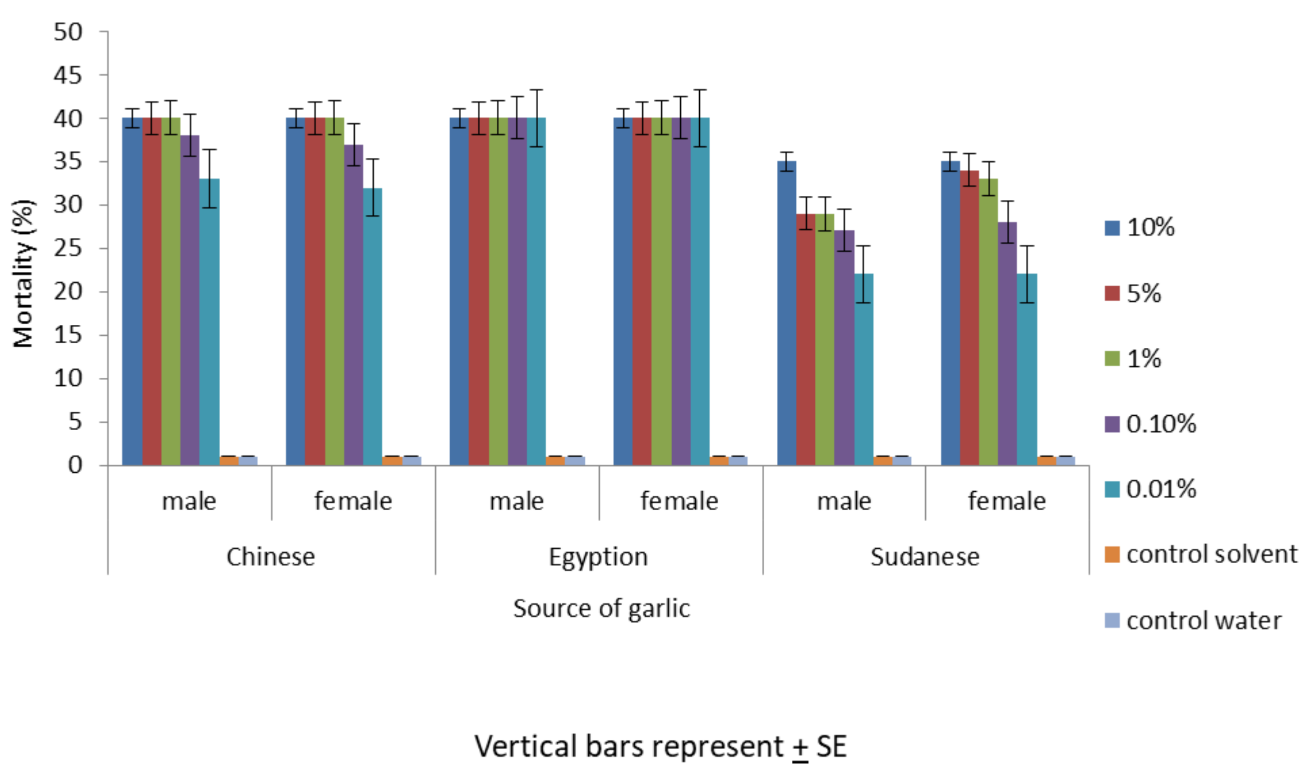

Figure 4. The 96 h mortality of male and female *Bruchidius incarnatus* exposed to garlic volatile oils.

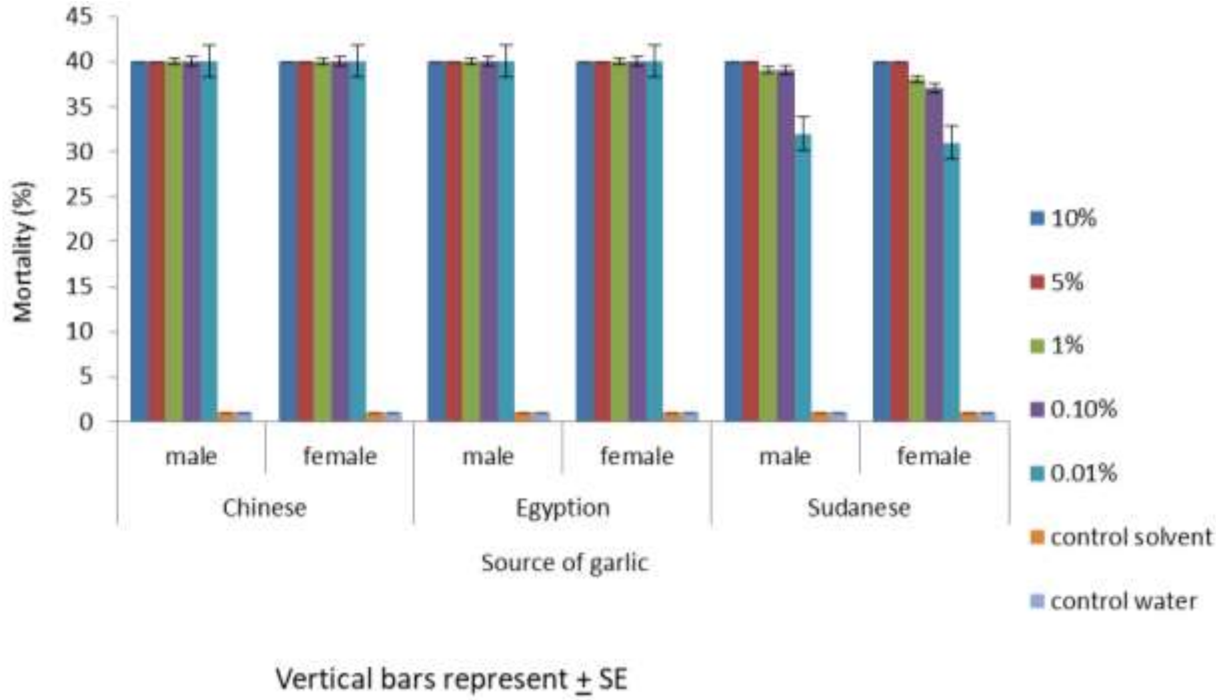

Figure 5. The 144 h mortality of male and female *Bruchidius incarnatus* exposed to garlic volatile oils.

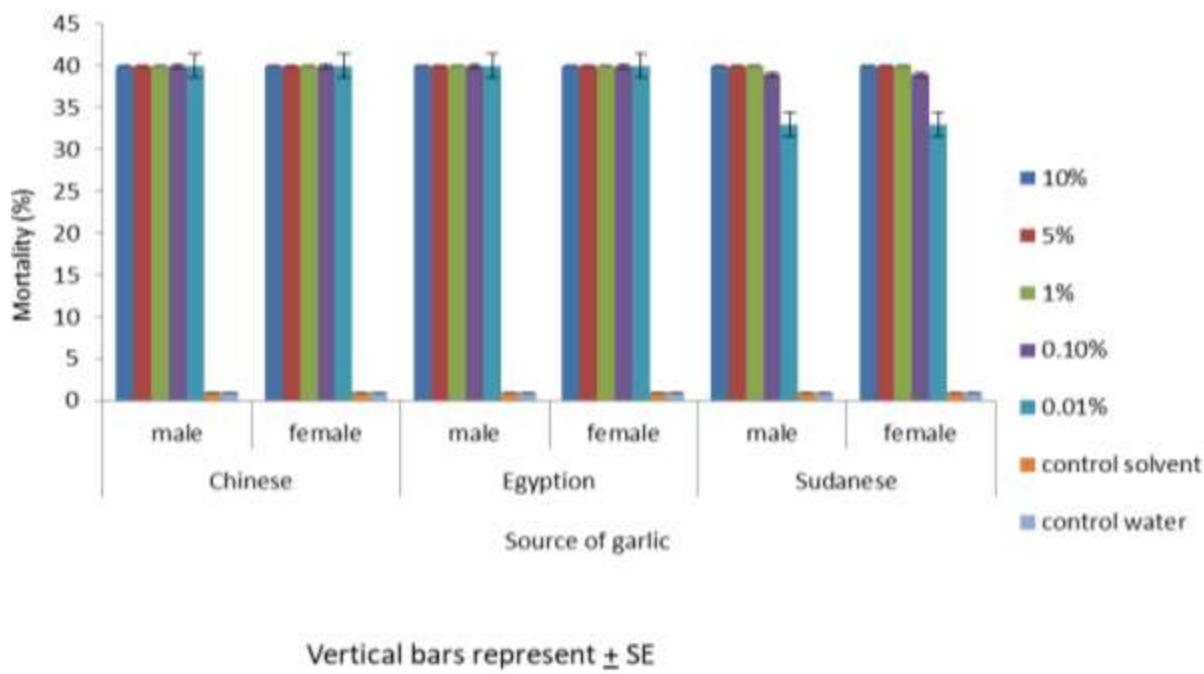

Figure 6. The 168 h mortality of male and female *Bruchidius incarnatus* exposed to garlic volatile oils.

were dose-related. Based on the mortality percentage the efficacy of the three oils can be ordered as follows: Chinese garlic volatile oil > Egyptian garlic volatile oil > Sudanese garlic volatile oil. These results are in accordance with Abdalla (2003) who studied the insecticidal effects of Chinese garlic essential oil on some major storage pests and reported that the garlic oil vapor can penetrate the jute sacs to exert its action. However, Taha (2007) reported that Egyptian garlic oil induced 100% mortality in *B. incarnatus* and *Callosobruchus maculatus* after 24 h exposure. However, Taha (2007) and Ahmed and Abdelbagi (2014) reported that Egyptian garlic oil induced 100% mortality in faba bean beetle, *B. incarnatus* and cowpea beetle, *C. maculatus* after 24 h exposure. Abdel-Tawab (2016) studied the insecticidal activity of some terpenes extracts from essential oils of garlic and mint against eggs, larva, and pupae of *Agrotis ipsilon*. He found that they caused high reduction in egg deposition (67.4%) and egg hatchability (69.4%) at sub-lethal concentration. Further, garlic essential oil reduced egg laying capacity in *Callosobruchus chinensis* adults either by fumigation or contact method as they reduced hatching rate in fumigated eggs (Mukesh, 2014).

Garlic volatile oil was reported to have insecticidal effect on test insect due to the inhibition of acetyl cholinesterase as reported by Bhatnagar and Pal (1974) and Singh and Singh (1996) or disruption of the cytochrome oxidase system of the mitochondria as reported by Dugravot et al. (2004).

Probit analysis

Data of various exposure periods showing uniform responses were subjected to probit analysis. From on the probit analysis, the 144 and 168 h exposure data for Egyptian oil and 168 h for Chinese oil at concentrations of 5 and 10% did not show a uniform response covering the entire dose range. The results are summarized subsequently.

Responses as related to dose

Dose-response data of male and female of *B. incarnatus* fumigated with oils from the three garlic cultivars are shown in Tables 1 and 2. The result of probit analysis indicated that both sexes of the test insects were sensitive to the vapor action of garlic oils as indicated by their low LD_{50} values. The LD_{50} values for 48 h exposure period to Chinese, Egyptian and Sudanese garlic oils were 0.05, 0.144 and 2.43% for males and 0.08, 0.56 and 6.1% for females following the same order. However, raising the exposure period to 96 h led to a significant reduction in the LD_{50} values to 0.03, 0.006 and 3.77% for males and 0.06, 0.03, and 3.77% for females, correspondingly. The male and female LD_{50} values of the Chinese oil reached a further much lower value after 144 h (0.007%). The slopes of the three oils ranged from 0.75 to 3.4 for the male and from 0.85 to 2.85 for female (Tables 1 and 2). Based on this, Chinese oil was more effective against the test insects as further indicated by its low LD_{50} value with narrow fudicial limits, narrow LD_{90}/LD_{50} ratio and low relative potency value followed by Egyptian and Sudanese oils (Tables 1 and 2).

These findings are consistent with those of Ahmed (1998), Wanyee et al. (1999), Abdalla (2003), Khiralla (2007), Taha (2007), Ahmed and Abdelbagi (2014) and Abdalla et al. (2017) who reported that increasing the pre-accumulation period of garlic oil and/or aqueous vapor in the exposure vessel (desiccators) resulted in better efficacy.

The population of the male and female of the test insects has relatively same homogeneity when tested against Chinese oil as indicated by their relatively similar slopes; but the population of the female was more homogenous compared to the male population tested against Egyptian oil as indicated by its high slope. However, the male population was relatively more homogenous compared to the female exposed to Sudanese oil as indicated by its high slope. The overall efficacy for both sexes followed the order: Chinese garlic volatile oil > Egyptian garlic volatile oil > Sudanese garlic volatile oil. The result showed that the female insects were more sensitive than males to Chinese oil; however, males are relatively more sensitive to Egyptian and Sudanese oils compared to females as indicated in their line slopes (Tables 1 and 2). This result agreed with that of Taha (2007) who stated that the population of *B. incarnatus* was more homogenous compared to *C. maculatus* with Egyptian garlic oil.

Responses as related to time

The mortality response which was uniformly distributed over the entire time range against the test insects was subjected to probit analysis. Based on this, the probit analysis was carried out at the concentrations of 0.01, 0.1, 1, 5 and 10% except for the data of 5 and 10% Chinese oil as it did not show a uniform response covering the entire dose range. Time-response of male and female of *B. incarnatus* fumigated with garlic volatile oils up to 168 h is shown in Tables 3 and 4. Results indicated that both sexes of the test insects were sensitive to the vapor action of the three garlic cultivars and their responses are time dependent as indicated by the LT_{50} values ranging between 79.73 and 17.47 h for males and 64.03 and 18.95 h for females. The result generally indicated that the male of the test insects was more sensitive to the three garlic cultivars oils vapor compared to the female as indicated by its low LT_{50} values, narrow fudicial limits and narrow LT_{90}/LT_{50} ratio (1.64-3.64 for male and 1.80-3.37 for female). The slopes of probit lines ranged from 0.17 to 0.44 for males and from 0.99 to 0.38 for females. The result indicated that the response of the female population was more homogenous to Chinese oil compared to the male population as indicated by the high slope of its probit line. However, both sexes have similar slopes when tested against Egyptian oil. The response of the males was more homogenous than that of the females to Sudanese

Table 1. Dose responses of the male *Bruchidius incarnatus* exposed to garlic volatile oils at different exposure periods (h).

Exposure time (h)	Source of garlic oil	Lethal dose (%)		Fudicial limits		LD_{90}/LD_{50} Ratio	χ^2 DF(3)	Slope	Relative potency
				Lower	Upper				
24	Chinese oil	LD_{10}	0.019	0.008	0.04	10.21	4.89	0.79	1
		LD_{50}	0.195	0.12	0.3				
		LD_{90}	1.99	1.15	4.17				
	Egyptian oil	LD_{10}	0.018	0.0007	0.08	638.02	0.47	2.19	57.49
		LD_{50}	11.21	3.7	95.285				
		LD_{90}	7152.2	459.19	4990699				
	Sudanese oil	LD_{10}	0.02	0.0017	0.075	256.48	0.86	1.88	22.67
		LD_{50}	4.42	2.26	21.56				
		LD_{90}	1390.13	178.44	99694.8				
48	Chinese oil	LD_{10}	0.04	0.001	0.009	12	2.49	0.84	1
		LD_{50}	0.05	0.03	0.08				
		LD_{90}	0.060	0.33	1.47				
	Egyptian oil	LD_{10}	$6×10^{-7}$	$6×10^{-8}$	0.0004	22837.99	1.81	3.4	2.8
		LD_{50}	0.144	0.012	0.58				
		LD_{90}	3286.4	120.12	175726696				
	Sudanese oil	LD_{10}	0.0008	$2*10^{-6}$	0.008	3243.8	0.913	2.74	48.6
		LD_{50}	2.43	0.78	15.07				
		LD_{90}	7887.96	355.6	27190058				
96	Chinese oil	LD_{10}	0.0018	$1*10^{-5}$	0.009	15.38	5.43	0.92	.72
		LD_{50}	0.026	0.003	0.07				
		LD_{90}	0.4	0.157	2.59				
	Egyptian oil	LD_{10}	$6×10^{-5}$	$2×10^{-5}$	0.001	94.17	2.78	1.54	1
		LD_{50}	0.0055	$5×10^{-5}$	0.028				
		LD_{90}	0.52	0.14	5.76				
	Sudanese oil	LD_{10}	0.011	$2×10^{-7}$	0.1707	348.42	1.031	1.98	685.45
		LD_{50}	3.77	0.44	2460.98				
		LD_{90}	1313.82	50.15	84.61				
144	Chinese oil	LD_{10}	0.0004	$2×10^{-5}$	0.003	16.11	0.64	0.75	1
		LD_{50}	0.007	$2×10^{-4}$	0.02				
		LD_{90}	0.11	0.04	1.14				
	Egyptian oil	LD_{10}	-	-	-	-	-	-	-
		LD_{50}	-	-	-				
		LD_{90}	-	-	-				
	Sudanese oil	LD_{10}	0.002	$14×10^{-5}$	0.03	207.67	0.94	1.11	61.43
		LD_{50}	0.43	0.04	1.67				
		LD_{90}	89.36	12.64	77584.62				
168	Chinese oil	LD_{10}	-	-	-	-	-	-	-
		LD_{50}	-	-	-				
		LD_{90}	-	-	-				
	Egyptian oil	LD_{10}	-	-	-	-	-	-	-
		LD_{50}	-	-	-				
		LD_{90}	-	-	-				
	Sudanese oil	LD_{10}	$57×10^{-6}$	$4×10^{-4}$	0.007	116.25	0.94	1.61	-

Table 1. Contd.

	LD_{50}	0.067	0.004	0.24
	LD_{90}	7.75	1.99	179.18

χ^2: Chi-square; DF: degrees of freedom; LD_{10}: lethal dose that induces 10% mortality of a certain population; LD_{50}: lethal dose that induces 50% mortality of a certain population; LD_{90}: lethal dose that induces 90% mortality of a certain population; (-) the data are not homogeneous therefore not subjected to probit analysis.

Table 2. Dose responses of the female *Bruchidius incarnatus* exposed to garlic volatile oils at different exposure periods (h).

Exposure time (h)	Source of garlic oil	Lethal dose (%)		Fudicial limits		LD_{90} /LD_{50} Ratio	χ^2 DF(3)	Slope	Relative potency
				Lower	Upper				
		LD_{10}	0.016	0.006	0.031				
	Chinese oil	LD_{50}	0.196	0.12	0.31	12.24	7.584	0.85	1
		LD_{90}	2.41	1.36	5.29				
		LD_{10}	0.014	$48*10^{-6}$	0.065				
24	Egyptian oil	LD_{50}	10.97	3.54	99.55	764.67	1.007	2.25	56.26
		LD_{90}	8386.78	493.61	7982436				
		LD_{10}	0.038	0.0036	0.12				
	Sudanese oil	LD_{50}	8.56	3.42	41.09	227.24	1.319	1.84	43.45
		LD_{90}	227.72	192187	192187				
		LD_{10}	0.005	0.001	0.01				
	Chinese oil	LD_{50}	0.08	0.05	0.14	17.76	4.88	0.98	1
		LD_{90}	1.48	0.78	3.64				
		LD_{10}	$1*10^{-4}$	$7*10^{-6}$	0.002				
48	Egyptian oil	LD_{50}	0.56	0.15	2.09	4464.12	1.67	2.85	7
		LD_{90}	2501.3	146.42	4537871				
		LD_{10}	0.002	$1*10^{-5}$	0.02				
	Sudanese oil	LD_{50}	6.1	1.85	61.87	2552.71	0.91	2.66	76.25
		LD_{90}	15581.8	592.68	89700333				
		LD_{10}	0.0026	$3*10^{-5}$	0.01				
	Chinese oil	LD_{50}	0.056	0.0079	0.15	21.56	2.46	1.04	2
		LD_{90}	1.2	0.46	6.41				
		LD_{10}	0.0018	$1*10^{-5}$	0.009				
96	Egyptian oil	LD_{50}	0.026	0.0028	0.09	15.13	5.43	0.92	1
		LD_{90}	0.04	0.16	2.59				
		LD_{10}	0.01	$16*10^{-7}$	0.17				
	Sudanese oil	LD_{50}	3.77	0.44	2460.98	348.42	1.02	1.98	125.7
		LD_{90}	1313.82	50.15	84.61				
		LD_{10}	$4*10^{-5}$	$2*10^{-7}$	0.003				
	Chinese oil	LD_{50}	0.007	$2*10^{-4}$	0.02	16.11	0.64	0.94	1
		LD_{90}	0.11	0.04	1.14				
		LD_{10}	-	-	-				
144	Egyptian oil	LD_{50}	-	-	-	-	-	-	-
		LD_{90}	-	-	-				
		LD_{10}	0.002	$14*10^{-6}$	0.03				
	Sudanese oil	LD_{50}	0.43	0.04	1.67	207.67	0.72	1.11	61.43
		LD_{90}	89.36	12.64	77584.62				

Table 2. Contd.

	Chinese oil	LD_{10}	-	-	-	-	-	-	
		LD_{50}	-	-	-	-	-	-	-
		LD_{90}	-	-	-				-
168	Egyptian oil	LD_{10}	-	-	-				
		LD_{50}	-	-	-	-	-	-	-
		LD_{90}	-	-	-				
	Sudanese oil	LD_{10}	$8*10^{-4}$	$36*10^{-5}$	0.0099				
		LD_{50}	0.12	0.008	0.41	22468.96	1.08	1.69	-
		LD_{90}	17.30	3.81	179.18				

χ^2: Chi-square; DF: degrees of freedom; LD_{10}: lethal dose that induces 10% mortality of a certain population; LD_{50}: lethal dose that induces 50% mortality of a certain population; LD_{90}: lethal dose that induces 90% mortality of a certain population; (-) the data are not homogeneous therefore not subjected to probit analysis.

Table 3. Time responses of the male *Bruchidius incarnatus* exposed to garlic volatile oils at different concentrations (%).

Concentration (%)	**Source of garlic oil**	**Lethal time (h)**		**Fudicial limits**		**LT_{90}/LT_{50} Ratio**	**χ^2 DF(3)**	**Slope**
				Lower	**Upper**			
0.01	Chinese oil	LT_{10}	31.89	55.73	37.13			
		LT_{50}	55.88	49.96	61.57	1.75	10.14	0.19
		LT_{90}	97.94	87.75	112.87			
	Egyptian oil	LT_{10}	29.59	23.99	34.22			
		LT_{50}	48.62	43.48	53.58	1.64	5.99	0.17
		LT_{90}	79.89	71.59	92.32			
	Sudanese oil	LT_{10}	28.43	19.29	36.46			
		LT_{50}	79.93	69.32	91.14	2.81	2.14	0.35
		LT_{90}	224.73	180.57	314.48			
0.1	Chinese oil	LT_{10}	16.03	10.82	20.74			
		LT_{50}	36.54	30.22	42.29	2.28	8.72	0.28
		LT_{90}	83.31	71.78	101.33			
	Egyptian oil	LT_{10}	23.78	18.73	28.06			
		LT_{50}	42.01	37.03	46.85	1.77	8.84	0.19
		LT_{90}	74.21	65.61	87.26			
	Sudanese oil	LT_{10}	25.49	18.41	31.77			
		LT_{50}	61.09	52.96	68.96	2.39	12.09	0.29
		LT_{90}	146.53	125.19	182.16			
1	Chinese oil	LT_{10}	8.56	2.48	13.39			
		LT_{50}	17.47	9.63	22.34	2.04	0.58	0.24
		LT_{90}	35.62	29.36	47.44			
	Egyptian oil	LT_{10}	16.79	11.85	21.08			
		LT_{50}	33.87	28.84	38.79	2.02	8.87	0.24
		LT_{90}	68.29	59.23	82.56			
	Sudanese oil	LT_{10}	13.14	7.43	18.71			
		LT_{50}	40.79	32.08	48.57	3.10	14.28	0.38
		LT_{90}	126.62	104.50	167.09			
5	Chinese oil	LT_{10}	-	-	-			
		LT_{50}	-	-	-	-	-	-
		LT_{90}	-	-	-			

Table 3. Contd.

	Egyptian oil	LT_{10}	15.72	10.28	19.84	1.96	12.19	0.23
		LT_{50}	30.78	25.66	35.38			
		LT_{90}	60.3	52.13	73.66			
	Sudanese oil	LT_{10}	9.002	3.99	14.33	3.64	16.15	0.44
		LT_{50}	32.76	23.26	40.93			
		LT_{90}	119.22	96.44	163.96			
10	Chinese oil	LT_{10}	-	-	-	-	-	-
		LT_{50}	-	-	-			
		LT_{90}	-	-	-			
	Egyptian oil	LT_{10}	12.74	7.69	16.94	2.02	5.57	0.24
		LT_{50}	25.71	20.21	30.26			
		LT_{90}	51.86	44.34	64.69			
	Sudanese oil	LT_{10}	8.91	4.17	13.83	3.19	7.75	0.39
		LT_{50}	28.39	20.09	35.57			
		LT_{90}	90.54	74.93	117.64			

χ^2: Chi-square; DF: degrees of freedom; LT_{10} ≡ Lethal time that induce 10% mortality of a certain population; LT_{50}≡ Lethal time that induce 50% mortality of a certain population; LT_{90} ≡Lethal time that induce 90% mortality of a certain population; (-) the data are not homogeneous therefore not subjected to probit analysis.

Table 4. Time responses of the female *Bruchidius incarnatus* exposed to garlic volatile oils at different concentrations (%).

Concentration (%)	Source of garlic oil	Lethal time (h)		Fudicial limits		LT_{90}/LT_{50} Ratio	χ^2 DF(3)	Slope
				Lower	Upper			
0.01	Chinese oil	LT_{10}	16.63	11.38	21.39	2.28	10.87	0.28
		LT_{50}	37.92	31.57	43.74			
		LT_{90}	86.44	57.54	105.06			
	Egyptian oil	LT_{10}	23.16	17.91	27.72	1.91	17.59	0.22
		LT_{50}	44.28	38.77	49.59			
		LT_{90}	84.66	74.5	99.95			
	Sudanese oil	LT_{10}	22.47	20.19	33.93	2.33	9.91	0.29
		LT_{50}	64.03	55.88	71.98			
		LT_{90}	149.24	128.04	184.35			
0.1	Chinese oil	LT_{10}	16.63	11.38	21.39	2.28	10.87	0.28
		LT_{50}	37.92	31.57	43.74			
		LT_{90}	86.44	75.54	105.06			
	Egyptian oil	LT_{10}	23.16	17.91	27.72	1.91	17.59	0.22
		LT_{50}	44.28	38.77	49.59			
		LT_{90}	84.66	74.5	99.95			
	Sudanese oil	LT_{10}	27.47	20.19	33.93	2.33	9.91	0.29
		LT_{50}	64.03	55.88	71.98			
		LT_{90}	149.24	48.04	184.35			
1	Chinese oil	LT_{10}	6.55	2.32	11.09	2.89	6.04	0.36
		LT_{50}	18.95	11.25	25.29			
		LT_{90}	54.83	44.69	70.38			
	Egyptian oil	LT_{10}	19.76	14.96	23.75	1.80	12.92	0.199
		LT_{50}	35.57	30.86	40.05			
		LT_{90}	64.04	56.04	76.79			

Table 4. Contd.

	Sudanese oil	LT_{10}	14.36	8.58	19.91	3.37	10.72	0.37
		LT_{50}	42.23	33.86	49.81			
		LT_{90}	142.19	103.44	160.94			
5	Chinese oil	LT_{10}	-	-	-	-	-	-
		LT_{50}	-	-	-			
		LT_{90}	-	-	-			
	Egyptian oil	LT_{10}	14.59	9.67	18.83	2.06	17.81	0.25
		LT_{50}	30.11	24.66	34.92			
		LT_{90}	62.12	53.46	76.19			
	Sudanese oil	LT_{10}	11.75	6.42	17.03	3.07	12.25	0.38
		LT_{50}	36.03	27.66	43.42			
		LT_{90}	110.47	91.73	143.76			
10	Chinese oil	LT_{10}	-	-	-	-	-	-
		LT_{50}	-	-	-			
		LT_{90}	-	-	-			
	Egyptian oil	LT_{10}	12.76	7.76	16.99	2.07	8.42	0.25
		LT_{50}	26.36	20.76	31.03			
		LT_{90}	54.48	46.58	67.73			
	Sudanese oil	LT_{10}	10.43	5.43	15.45	3.03	8.31	0.38
		LT_{50}	31.57	23.45	38.63			
		LT_{90}	95.57	79.65	122.93			

χ^2: Chi-square; DF: degrees of freedom; LT_{10} ≡ Lethal time that induce 10% mortality of a certain population; LT_{50}≡ Lethal time that induce 50% mortality of a certain population; LT_{90} ≡Lethal time that induce 90% mortality of a certain population; (-) the data are not homogeneous therefore not subjected to probit analysis.

oil as indicated by the high slope of their probit line. Chi-square values were small indicating good execution of the experiments. Generally, the result showed that the male insects were more sensitive to the three oils compared to the female insects as indicated by their low line slopes (Tables 3 and 4).

The knockdown and recoveries among the insects fumigated with garlic essential oil were reported by Ahmed (1998), Abdalla (2003), Khiralla (2007) and Taha (2007). The significance of watching recoveries of treated insects is essential for better reading of the end-point mortality as evaluated by Beard (1949) and Winks (1982). The end-point mortality was defined by Beard (1949) as an observation period in which treated individuals either die or recover (that is, the poison has exerted its full toxic effect). The results of the current study indicated that no significant recoveries were observed in the different fumigation trails within the various exposure periods (7 days). These findings are in accordance with Abdalla (2003) who reported that the adult of *C. maculatus* did not recover after four days and Khiralla (2007) who stated that the adult of *B. incarnatus* did not recover after five days following the end of the exposure period. This may indicate that the Bruchid beetles were very sensitive to garlic oil vapor and the effects were long-lasting.

Oviposition inhibitory assay

A number of eggs laid per female are as shown in Figure 7. Results showed that fewer eggs were laid after a week of exposure to Chinese garlic volatile oil which recorded lowest number of eggs with a mean of 3.25 at the highest concentration 10%; while the highest numbers of eggs, with mean of 22.75 were observed at the lowest concentration of 0.01. The result was significantly different compared to the control set which recorded average number of eggs of 51.75 and 50.25, respectively. Also, there are significant differences among the different treatments. While in Egyptian oil the lowest number of eggs (4.5) was recorded at the highest concentration (10%) and the highest number of eggs (22.75) was observed at the lowest concentration (0.01%). Results showed that all the three oils induced a significant reduction in the number of eggs laid by exposed females compared to the controls and effects were dose-dependent (Figure 7). Generally, Chinese oil

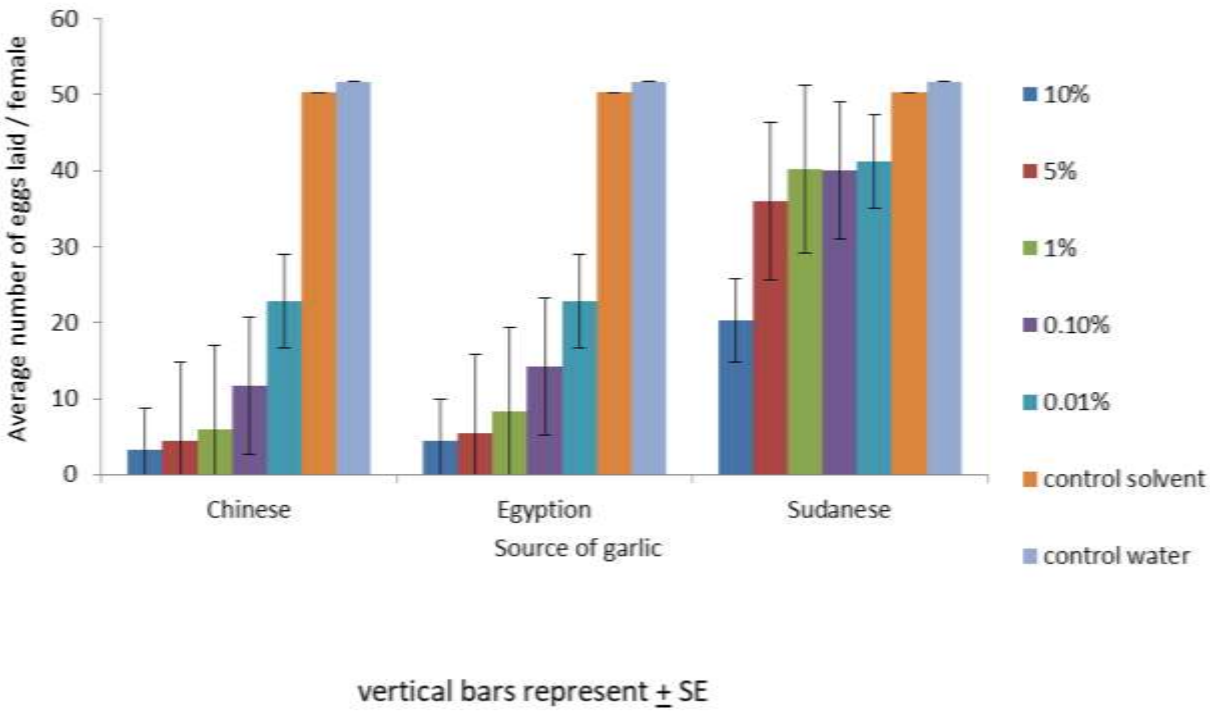

Figure 7. Average number of eggs laid per female *Bruchidius incarnatus* exposed to garlic volatile oils for a week.

was the most effective followed by Egyptian and Sudanese oil.

Table 5 shows the log-dose of oviposition responses of *B. incarnatus* exposed to garlic oils. The result indicated that the test insect is more sensitive to Egyptian garlic oils compared to the Sudanese oil with LD_{50s} of 36.39% for the Sudanese garlic oil and 1.88×10^{-4}% for the Egyptian garlic oil. The corresponding LD_{90s} values are 0.0015 and 5.219×10^{-9}%. The slopes of the inhibition regression line were steep and positive, indicating the homogeneity of the population or fast action of garlic oils. Chi-square values ranged from 0.44 to 12.45.

This result agrees with Singh and Singh (1996) who reported that allyl disulfide, a component from garlic oil, decreased the fecundity and adult emergence of *Rizopertha dominica*. The results are also in accordance with Ho et al. (1996), who demonstrated that garlic oil is effective in reducing F1 progeny production in both *Tribolium castaneum* and *Sitophilus zeamais*, and is toxic to the eggs, adults, and larvae of *T. castaneum.*

The suppression in egg lying induced by garlic volatile oils may be due to their actions on some hormones or due to harmful effects on the reproductive system of the test insects or behavioral effects by obscuring the recognition of host. It was also observed that there was a decrease in egg laying with the increase in concentrations used. These findings agreed with Fagger (1999) who found that Usher *Calotropis procera*, Neem kernel *Azadirachta indica* and garlic *A. sativum* (oil and powder) have negative effects on the fecundity, hatchability and adult emergence of *B. incarnatus* (Boh.).

Ovicidal assay

The ovicidal effects of the three oils were measured by the hatchability of the fumigated eggs as displayed in Figure 8. Volatile oils of the three garlic cultivars had different effects on the eggs of *B. incarnatus*. The concentrations used were 0.01, 0.05, 0.1, 1, 2, 10 and 20%. The upper dose (20%) in all the three oils recorded 0 hatchability and caused significant ovicidal effect compared to the rest of the concentrations. The average hatchability of eggs recorded at the lowest concentration of 0.01% was 31, 32 and 34% for Chinese, Egyptian and Sudanese oils, respectively. Results showed that all the three oils induced a significant reduction in the hatchability of fumigated eggs compared to the controls (49.75%) and effects were dose-dependent (Figure 8). Generally, Chinese oil was the most effective followed by Egyptian and Sudanese oil.

Dose-response data of the fumigated eggs are shown in Table 6. The result of probit analysis confirms the sensitivity of fumigated eggs to the vapor action of the three garlic cultivars as indicated by their LD_{50s} values of 0.025, 0.034 and 0.21% for Chinese, Egyptian and Sudanese oil, respectively. Slopes of their probit lines are 0.46, 0.58 and 0.93 following the same order. The test eggs were more sensitive to Chinese oil as indicated by their low LD_{50} value with narrow fudicial limits and narrow

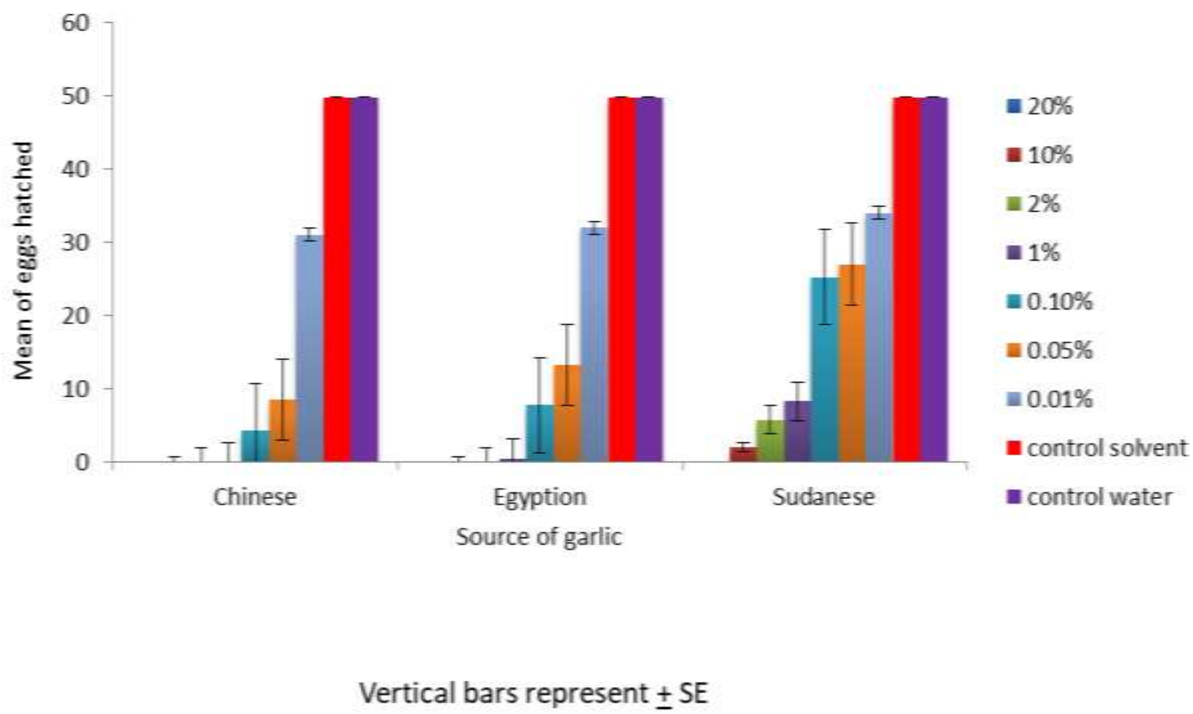

Figure 8. Hatchability of eggs of *Bruchidius incarnatus* exposed to garlic volatile oils.

Table 5. Dose response of eggs of faba bean beetle *Bruchidius incarnatus* exposed to garlic volatile oils.

Source of garlic oil	Lethal dose (h)		Fudicial limits		LT_{90}/LT_{50} Ratio	χ^2 DF(3)	Slope	Relative potency
			Lower	Upper				
	LD_{10}	0.007	0.004	0.009				
Chinese oil	LD_{50}	0.025	0.02	0.03	3.6	1.42	0.49	1
	LD_{90}	0.09	0.08	0.13				
	LD_{10}	0.006	0.004	0.009				
Egyptian oil	LD_{50}	0.034	0.03	0.04	5.59	2.83	0.58	1.36
	LD_{90}	0.19	0.15	0.26				
	LD_{10}	0.014	0.008	0.022				
Sudanese oil	LD_{50}	0.21	0.16	0.27	15.48	5.27	0.93	8.4
	LD_{90}	3.25	2.39	4.66				

χ^2: Chi-square; DF: degrees of freedom; LD_{10} ≡ Lethal dose that induce 10% mortality of a certain population; LD50≡ Lethal dose that induce 50% mortality of a certain population; LD_{90} ≡ Lethal dose that induce 90% mortality of a certain population.

LD_{90}/LD_{50} ratio followed by Egyptian oil and lastly Sudanese oil. The population of the test eggs was more homogenous in response to Sudanese oil as indicated by its high slope followed by Egyptian and Chinese oils. Chi-square values were small indicating good execution of the experiments.

Similar results were obtained by Neveu et al. (1997) who reported that garlic compounds may toughen the structure of the egg, preventing hatching in a way similar to that of dehydration. Mukesh (2014) claimed that the garlic essential oil significantly reduced the hatching rate in *C. chinensis* eggs when fumigated. He also indicated that the mean number of eggs hatched per 25 eggs was reduced to 20.33 (81.32%) and 5.16 (20.64%) when fumigated with lowest and highest concentration, respectively as compared to 22.33 (89.32) eggs hatched

Table 6. Dose response of eggs laid per female *Bruchidius incarnatus* exposed to garlic volatile oils for a week.

Source of garlic oil	Lethal dose (h)		Fudicial limits		LT_{90}/LT_{50} Ratio	χ^2 DF(3)	Slope	Relative potency
			Lower	Upper				
Chinese oil	LD_{10}	1.9276	0.6778	11.1169	0.00158	0.448	2.17	16.78
	LD_{50}	0.003156	0.000188	0.01317				
	LD_{90}	0.000005	$7.651*10^{-9}$	0.000106				
Egyptian oil	LD_{10}	6.7955	1.2224	984.9954	0.0000026	3.556	1.35	1
	LD_{50}	0.000188	$3.73*10^{-9}$	0.003787				
	LD_{90}	$5.219*10^{-9}$	$3.766*10^{-20}$	0.000004				
Sudanese oil	LD_{10}	847633.5	6324.755	$4.134*10^{-12}$	0.000042	12.45	3.4	193.6
	LD_{50}	36.3968	6.5498	3747.522				
	LD_{90}	0.001563	0.000001	0.01680				

χ^2: Chi-square; DF: degrees of freedom; LD_{10} ≡ Lethal dose that induce 10% mortality of a certain population; LD50≡ Lethal dose that induce 50% mortality of a certain population; LD_{90} ≡ Lethal dose that induce 90% mortality of a certain population.

in the control.

The ovicidal effects of volatile from garlic on eggs of four cotton insect pests, *Earias vittella* (F.), *Dysdercus koenigii* (F.), *Spodoptera litura*, and *Helicoverpa armigera* (Hubert) have been reported by Gurusubramanian and Krishna (1996). HO et al*.* (1997) reported that volatile oil from fresh garlic cloves (obtained by steam distillation) can cause dose-related mortality in eggs, larvae, and adult of *T. castaneum* with eggs being more susceptible than adult and larvae. Further, Ibrahim (2000) studied the ovicidal effect of *Ocimum basilicum* on the eggs of faba bean beetle, *B. incarnatus* and reported that the powder and crude extracts of the test plant reduced the oviposition rate and had ovicidal effects against the test eggs.

In conclusion, the current results clearly demonstrated the efficacy of fumigant action of garlic volatile oils against faba bean beetle, *B. incarnatus* inducing significant mortality, oviposition inhibitory and ovicidal effects. As indicated elsewhere, the active principle in garlic oil is a simple sulfur-containing compound; therefore similar analogs could easily be synthesized and applied as both fumigant and contact insecticide. The expected low mammalian toxicity of garlic, being widely used for culinary purposes, can add to its merits.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Screening of allelopathic activity of common weed species occurring in agricultural fields

Ewon Kaliyadasa* and Sadeeka Layomi Jayasinghe

Department of Export Agriculture, Faculty of Animal Science and Export Agriculture, Uva Wellassa University, Badulla, Sri Lanka.

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The investigation of allelopathic effects of different plant species is important to prevent serious crop losses that would arise. The usage of such materials as mulch, cover crops or residues will be useful to implement in integrated weed management strategies of agricultural fields. Therefore, this study was conducted to screen the allelopathic effect of selected plant species using seedlings growth of lettuce (*Lactuca sativa* L.) as an indicator. The sandwich method was used and 20 mg of dried plant material, resembling the leaf litter, from 27 species were placed in petri dishes. Twenty-eight treatments including the control treatment were arranged in a Completely Randomized Design (CRD). On the fifth day after the establishment of lettuce seeds, hypocotyl length (cm), radicle length (cm) and total height (cm) of seedlings were measured. The inhibitory percentage was calculated and a dendrogram with single linkage was developed. Results revealed that the lowest hypocotyl and radicle lengths with the highest inhibitory percentage were recorded for *Ageratum conyzoides, Cassia occidentals and Clidemia hirta* when compared to the control treatment ($p<0.05$). Weed species expressed varying degree of inhibitory effects on growth performances of lettuce seedlings. Further studies need to be carried out to explore the effects of allelopathy on crop plants.

Key words: Inhibitory percentage, lettuce, seedling growth, hypocotyl, radicle.

INTRODUCTION

Allelopathy is considered as an interference mechanism available to plants, which release chemicals that influence the growth and development of neighboring plants in both natural and agricultural ecosystems. This process happens through leaching, root exudation, volatilization, residue decomposition and other means by creating direct and indirect effects on the adjacent micro-environment due to (Khanh et al., 2007) chemical substances released by plants (Einhelling, 2008).

Allelochemicals results in harmful effects and is categorized into two, true allelopathy and functional allelopathy (Duke, 2015). The true allelopathy is the release of substances that are toxic in nature from its origin as they are produced in plants (Duke, 2015). Functional allelopathy is the release of toxic substances, which are resultants of transformation by microorganism (Inderjit et al. 2002; Jabran and Farooq, 2013). These chemicals accrue and persevere for a substantial time in the plant, thereby causing significant interference on growth and development of neighboring plants (Einhelling, 2008) which can be either a crop or a weed. With considerable work conducted in past decades, the

*Corresponding author: E-mail: ewon101k@yahoo.com. Tel: +94718310540.

presence of inhibitory compounds in a wide array of plant types and its parts at varying concentrations were investigated and with significant attention being paid to allelopathy (Inderjit et al. 2002) and its related effects.

As the understanding of the related mechanisms of these allelochemicals in plants grew, significant attention was given to the potential use of allelopathy as a weed management strategy in achieving sustainable agriculture, with weed control a challenging task, particularly in organic farming (Dayan et al., 2009). Integration of allelopathy into natural and agricultural management systems might help to reduce the use of synthetic agrochemicals. Ultimately, it will reduce the negative effects on the environment, especially pollution. Exploring crop allelopathy against weeds might be a useful strategy to eliminate problems created by synthetic herbicides (Anjum and Bajawa, 2005). Recently, several crops and weeds showed explicit weeds suppressive ability and with potential use in agricultural fields, by exuding allelochemical compounds either from living plants or from decomposing residues (Xuan et al., 2006). Further, this positive effect on weed control will definitely be helpful in introducing potential allelopathic crops into crop rotation programmes, either as a cover crop or mulch to smother crops, or as a green manure in sustainable agricultural practices. This is a very positive trend in agriculture, which could apply for the benefit of human beings by reducing chemical usage. Further, it helps to minimize severe health problems that could be caused due to unsafe handling of agrochemicals (Belz, 2007).

Moreover, the concept of allelopathy can be utilized effectively to produce eco-friendly natural herbicides (Sodaeizadeh et al., 2009). Therefore, knowledge of allelopathic properties of crops or weeds will be beneficial to farmers who adopt different agronomic practices, which help to reduce the cost of production by eliminating the cost incurred for agrochemicals. Furthermore, allelochemicals as natural herbicides would help to mitigate the hitches created by synthetics. In addition, specific allelopathic chemicals enable the development of highly specific herbicides. In cultural and agronomic practices such as mulching, use of residues and cover crops, the specific allelochemicals in plants could also be exploited for weed management. The investigation into and development of new transgenic crop cultivars by incorporating allelopathic properties would reduce usage of synthetic herbicides causing benefits to the environment as well as to agricultural systems (Bhadoria, 2011). As reported by Duke et al. (2015), allelochemicals help to mitigate soil sickness and improve soil properties through controlling ion exchange capacity, organic matter, reactive mineral surfaces, inorganic ions, and also micro and macro fauna and flora of soil. More importantly, information on plant allelopathy could prevent serious crop damage that would arise due to buried biomass in soil in previous years as mulch, cover crop or residues (Jabran et al., 2015). The use of allelopathic traits in crops or weeds will provide a comprehensive understanding for further effective use in the agriculture sector. Particularly, allelopathy will create portentous opportunity to implement integrated weed management strategies and will be exploited as natural weapons to alleviate serious weeds problems in the agriculture sector.

During past decades, the suppressive ability of allelochemicals in weeds has drawn significant attention. However, the specific plant species and its unique responses were not clearly studied yet. Therefore, the focus of this study was to screen the allelopathic effect of naturally grown weed species that commonly occur in agricultural fields. The objective of the study was to study the allelopathic effect of selected common weed species on lettuce seedlings.

MATERIALS AND METHODS

Twenty-seven weed species were randomly collected from Glen Alpine Estate and University Farm (6.981° North, 81.077° East; 1120 m amsl altitude) and laboratory analysis were conducted at Uva Wellassa University. The study location belongs to Agro-ecological region of IM1a (Intermediate zone, mid country). Whole plants of twenty-seven weed species (Table 1) were collected fresh, placed in polythene bags separately, and then taken to the laboratory. The whole plants were cleaned and dried in an oven (VT 6025) at 40°C temperature for 48 h. After appropriate drying, plant materials of each species were kept in airtight plastic bags until further use. The dried plant samples were subjected to analysis for its allelopathic effects using the sandwich method, as described by Fujii et al. (2003). Rapido 344 variety of Lettuce (*Lactuca sativa* L.), 20 mg of dried plant material of each weed species were placed in autoclaved petri dish resembling the leaf litter. Five milliliters of 0.5% w/v agar was poured in two layers on dried plant material and three lettuce seeds were placed in each petri dish. Afterwards, the petri dishes were placed in the growth room under complete darkness at 25°C. In order to neutralize evaporation and changes to the composition of plant material, the caps of the petri dishes were sealed firmly.

Twenty-eight treatments (Table 1) including the control with seven replicates were arranged in a Completely Randomized Design (CRD). On the fifth day, hypocotyl length (cm), radicle length (cm) and total height (cm) of seedlings were measured. The percentage of inhibition in germination of lettuce seedlings was determined using the following formula as reported by Chung et al. (2001).

Inhibition percentage (%) = [(Total length of sample-Total length of control)/ Total length of control] ×100

Data were statistically analyzed in a one-way ANOVA procedure and significant differences were calculated using the Tukey test at 95% confidence intervals in Minitab 18 software. A dendrogram was created using scores of the mean values of hypocotyl length, radicle length and inhibitory percentage.

RESULTS

Table 1 shows the result of the effect of leaf litter of different weed species on the growth of lettuce seedlings

Table 1. Allelopathic effect of leaf litter of different weeds on growth and development of lettuce seedlings.

S/N	Botanical name	Plant family	Hypocotyl length (cm)	Radicle length(cm)	Inhibitory percentage(%)
1	*Control*	-	1.93±0.37^{a}	3.16±0.52^{a}	-
2	*Ageratum conyzoides*	Asteraceae	0.42±0.26^{d}	0.43±0.33^{fg}	83.16^{fg}
3	*Sphagneticola trilobata*	Asteraceae	0.86±0.52^{bcd}	1.14±0.90^{bcdefg}	60.63^{bcdefg}
4	*Chromolaena odorata*	Asteraceae	0.82±0.43^{bcd}	1.57±0.67^{bcdef}	52.97^{bcdefg}
5	*Crassocephalum crepidioides*	Asteraceae	1.48±0.72^{ab}	1.84±1.16^{bc}	34.60^{abc}
6	*Erigeron sumatrensis*	Asteraceae	1.28±0.45^{abcd}	1.71±0.6^{bcde}	41.16^{bcd}
7	*Bidens pilosa*	Asteraceae	1.06±0.71^{abcd}	1.7±0.99^{bcde}	45.80^{bcdef}
8	*Solanum americanum*	Solanaceae	0.90±0.44^{bcd}	0.61±0.37^{defg}	70.25^{cdefg}
9	*Cardiospermum halicacabum*	Sapindaceae	0.77±0.64^{bcd}	1.10±0.62^{bcdefg}	63.25^{bcdefg}
10	*Sida acuta*	Malvaceae	1.41±0.28^{ab}	1.36±0.17^{bcdefg}	45.54^{bcdef}
11	*Cleome aspera*	Capparaceae	1.12±0.57^{abcd}	1.13±0.76^{bcdefg}	55.60^{bcdefg}
12	*Cassia occidentalis*	Fabacea	0.46±0.39^{d}	0.5±0.41^{efg}	81.19^{efg}
13	*Cassia tora*	Fabacea	1.32±0.54^{abcd}	1.47±0.37^{bcdefg}	45.10^{bcdef}
14	*Mimosa pudica*	Fabacea	1.42±0.28^{ab}	1.23±0.7^{bcdefg}	47.73^{bcdefg}
15	*Cyperus rotundus*	Cyperaceae	1.52±0.69^{ab}	2.91±0.89^{ab}	26.90^{ab}
16	*Achyranthus aspera*	Amaranthaceae	1.30±0.37^{abcd}	1.62±0.84^{bcdef}	42.48^{bcde}
17	*Urena lobata*	Amaranthaceae	1.17±0.54^{abcd}	1.36±0.67^{bcdefg}	50.35^{bcdefg}
18	*Aerva lanata*	Amaranthaceae	1.04±0.44^{abcd}	0.91±0.55^{cdefg}	61.50^{bcdefg}
19	*Amaranthus viridis*	Amaranthaceae	1.00±0.27^{bcd}	0.78±0.2^{cdefg}	65.00^{bcdefg}
20	*Stachytarpheta urticaefolia*	Verbenaceae	1.11±0.64^{abcd}	1.72±1.04^{bcd}	44.20^{bcdef}
21	*Lantana camara*	Verbenaceae	0.96±0.68^{bcd}	1.13±0.49^{bcdefg}	58.88^{bcdefg}
22	*Phyllanthus debilis*	Euphorbiaceae	1.40±0.78^{abc}	1.83±0.92^{bc}	36.40^{abcd}
23	*Commellina diffusa*	Commelinaceae	1.03±0.42^{abcd}	1.5±0.58^{bcdef}	50.13^{bcdefg}
24	*Cuscuta reflexa*	Convalvulaceae	0.74±0.45^{bcd}	0.52±0.25^{defg}	75.07^{defg}
25	*Clidemia hirta*	Melastomataceae	0.49±0.23^{cd}	0.28±0.12^{g}	84.91^{g}
26	*Osbeckia octandra*	Melastomataceae	1.06±0.52^{abcd}	1.27±0.80^{bcdefg}	54.29^{bcdefg}
27	*Ocimum sanctum*	Lamiaceae	0.89±0.52^{bcd}	1.64±0.91^{bcdef}	50.13^{bcdefg}
28	*Hyptis suaveolens*	Lamiaceae	1.24±0.46^{abcd}	1.29±0.7^{bcdefg}	50.13^{bcdefg}

Note: Means with same superscript in a column are not significantly different from each other while means with different superscript are significantly different at $p<0.05$.

on agar medium. There was a significant difference in the effect of allelopathy of weed species on growth and development of hypocotyl, radicle and percentage inhibition of lettuce seedlings. Many weed species tested showed significantly different responses on inhibitory percentage, hypocotyl and radicle lengths of lettuce plant compared to control in the experiment (Table 1). The highest hypocotyl and radicle lengths were recorded in control treatment (1.93±0.37 and 3.16±0.52 cm, respectively) *family* without any leaf litter. Except for *Cyperus*

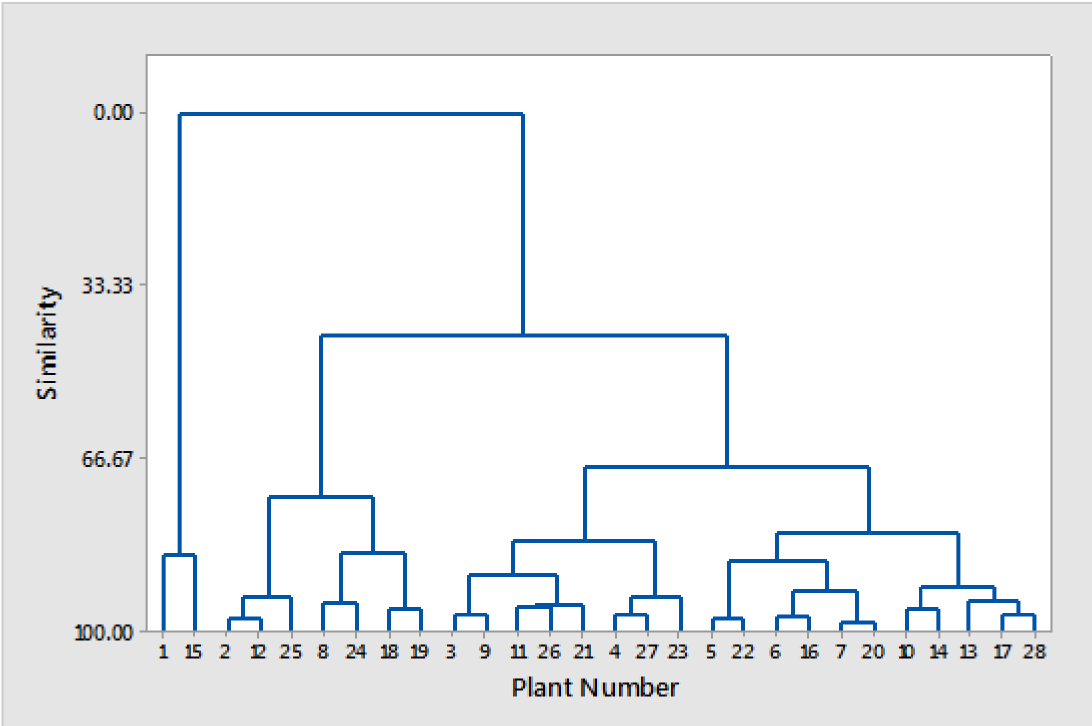

Figure 1. Dendrogram with single linkage and euclidean distance on effect of allelochemicals of different common weed species on hypocotyl length (cm) and radicle length (cm) of lettuce seedlings.

rotundus, all other species showed a significant difference in the development of radicle length compared to control treatment (p<0.05).

Conspicuously two main clusters were formed in the dendrogram according to the varying degree of allelopathic effect of different plant species on seedling growth of lettuce (Figure 1). *C. rotundus* has shown very similar results as in control treatment where no leaf litter was used, with values of 1.52±0.69 cm hypocotyl length and 2.91±0.89 cm of radicle length (Table 1). In agreement with the above results, both control treatment and *C. rotundus* are in the same cluster representing its' close similarity on observed variables in the present study (Figure 1).

The lowest hypocotyl and radicle lengths with the highest inhibitory percentage were recorded in *Ageratum conyzoid* (0.42±0.26 cm, 0.43±33 and 83.16%), *Cassia occidentals* (0.46±0.39 cm, 0.5±41 and 81.19%) and *Clidemia hirta* (0.49±0.23 cm, 0.28±0.12 cm and 84.91%) which were significantly different from control treatment (p<0.05) (Table 1). Therefore, data suggests that there is a strong effect of allelopathy on growth and development of lettuce seedlings above three weed species (Figure 2), which commonly occur in cropping lands. Furthermore, these three species were grouped in one cluster (Figure 1) showing its similarity compared to the control as well as other weed species.

According to the results, it is apparent that leaf litter of *Solanum americanum* and *Cuscuta reflexa* exhibit significant effects on hypocotyl length (0.90±0.44 cm, 0.74±0.45 cm) and radicle lengths (0.61±0.37 cm, 0.52±0.25 cm), respectively with the second highest inhibitory percentages (70.25 and 75.07%) recorded in the study and appeared in the same sub cluster (Figure 1). Exhibiting close similarity to the hypocotyl and radicle lengths, *Aerva lanata* and *Amaranthus viridis*, which belongs to family of Amaranthaceae, have formed a sub-level in the same sub-cluster with *Solanum americanum* and *Cuscuta reflexa* as shown Figure 1.

Other weed species were grouped into another sub cluster, which consists of a number of ancillary sub clusters. Based on varying degree of similarity as shown in Figure 1. *Sphagneticola trilobata, Cardiospermum halicacabum, Cleome aspera, Osbeckia octandra, Lantana camara, Chromolaena odorata, Ocimum sanctum* and *Commellina diffusa* are included in the same sub cluster, but connected with three separate limbs. Above mentioned weed species representing two limbs of the same sub cluster have shown a significant difference in radicle lengths and inhibitory percentage of lettuce seedlings compared to the control (p<0.05). However, there was no significant difference in hypocotyl length in *Osbeckia octandra* and *Cleome aspera* (1.06±0.52 cm and 1.12±0.57 cm, respectively) compared to the control treatment. *Chromolaena odorata, Commellina diffusa* and *Ocimum sanctum* in the same

Figure 2. Three common weed species with high allelopathic effect (a) *A. conyzoides* (b) *C. occidentalis* (c) *C. hirta.*

level of the third limb of the above sub cluster have shown a negative effect on the growth of lettuce seedlings. According to the results generated by the Tukey test, there was a significant difference between above three weed species and the control treatment. It resulted statistically in different radicle lengths and inhibitory percentage except the hypocotyl length of *Commellina diffusa,* as it is statistically similar to the response of control treatment ($p<0.05$).

Weed species of *Crassocephalum crepidioide, Phyllanthus debilis, Erigeron sumatrensi, Achyranthus aspera, Bidens pilosa* and *Stachytarpheta urticaefolia* formed another sub cluster indicating close similarity as shown in Figure 1. *Crassocephalum crepidioides* of Asteraceae family and *Phyllanthus debilis* of Euphorbiaceae family have similar responses on inhibitory percentage, hypocotyl and radicle length of lettuce seedlings than that of other weed species of that sub cluster and thus grouped into a same sub-level. *Erigeron sumatrensis*, *Achyranthus aspera, Bidens pilosa* and *Stachytarphet aurticaefolia* exhibit statistically similar results (Table 1) and in the same limb of the sub cluster. The group of *Sida acuta, Mimosa pudica, Cassia tora, Urena lobata* and *Hyptis suaveolens* rested in a separate sub cluster laid far from the control compared to other weed species.

Diverse responses have resulted in weed species, which belong to different plant families in the present study. Six-tested weed species representing Asteraceae family (*Ageratum conyzoides, Sphagneticola trilobata, Chromolaena odorata, Crassocephalum crepidioides, Erigeron sumatrensis* and *Bidens pilosa*) exhibit diverse effects on the overall performance of lettuce seedlings. It showed a wide range of inhibitory action compared to the control treatment, which varied from 34.60 to 83.16% (Table 1) and subjected to fall in different sub clusters in the dendogram analysis (Figure 1).

The present study shows that leaf litter of four species of Amaranthaceae family (*Achyranthus aspera, Urena lobata, Aerva lanata and Amaranthus viridis*) resulted in non-significant responses for length of hypocotyl, radicle and inhibition percentage. However there was a significant difference between four species mentioned above and the control treatment only for radicle length and inhibitory percentage of seedlings (at $p<0.05$) (Table 1). However, no significant difference in hypocotyl length compared to control treatment was observed, except for *Amaranthus viridis.*

According to the results, there was a significant difference between *Cassia occidentalis* and other tested weed species of Fabaceae family (*Cassia tora* and *Mimosa pudica*) on all three observations and the inhibitory percentage ranged from 45.10 to 81.19% as observed in the emergence of lettuce seedlings. There was no significant difference between *Clidemia hirta* and *Osbeckia octandra* weed species of Melastomataceae family on the observed parameters of lettuce seedling growth ($p>0.05$). A similar response was exhibited in members of Verbanaceae and Lamiaceae families as there was no significant difference between two weed species of each family (Table 1) and formed part of separate sub clusters in the dendogram (Figure 1).

Comparable allelopathy effects were accrued by weed species belonging to Solanaceae and Convalvulaceae families to the observed parameters on seedling growth of the study and formed the same level of similarity in the dendogram (Figure 1).

DISCUSSION

Effect of leaf litter of weed species belong to different plant families on growth of lettuce seedlings in sandwich method was studied. The present study found that leaf litter of the *Ageratum conyzoides* of the family Asteraceae, *C. occidentalis* from Fabacea and *Clidemia hirta* of the family Melastomataceaeca resulted in over 80% of inhibition of the growth of lettuce seedlings and ranked first among the weed species tested in this study.

Ageratum conyzoides is a tropical herb that is very common in Asia and called as Billy Goat weed, which has allelopathy potential in nature. In Sri Lanka, *A. conyzoides* is traditionally used as green manure and raw material in compost production. Some researchers reported that the ability of green manure of *A. conyzoides* to upsurge crop yields and generally it is intercropped as understory with perennial crops to subdue weeds and control of pests (Kong et al. 1999). *A. conyzoides* performs to be a valued agricultural resource, as it comprises a wide range of secondary metabolites including flavonoids, chromenes, benzofurans and terpenoids (Kong et al., 1999). Among these secondary metabolites, some are allelochemicals, which impede the growth of other organisms (Okunade, 2002). *A. conyzoides* can biosynthesize and release non-volatile allelochemicals into the soil. Thus, it inhibits the growth of other plants and microorganisms in soil. It was found that polymethoxy flavones, ageratochromene and its analogues are rare in natural products but they have been discovered in *A. conyzoides* (Okunade, 2002). In fact, *A. conyzoides* possesses the ability of applying in different aspects of agriculture, potentially of allelopathy in agro-ecosystems. The findings of the present study it suggests the negative affects seedling growth. Hence, the use of these plant materials in compost production might affect the early growth of seedlings and the farmers can expect positive effects. However, with all these studies, it has shown the possibility of using this plant species to control other competitive weeds in croplands as an alternative approach for improved weed management especially in organic farming.

C. occidentalis L. is a nitrogen-fixing plant belonging to the subfamily Caesalpinioideae of the family Fabaceae. Many studies have established to demonstrate the allopathic inhibition by different plant parts, residue and aqueous extracts (Gurib-Fakim, 2006). Inhibition of growth of lettuce seedlings in the present study might be due to the above stated allelopathic influence of chemicals released by leaf litter of *C. occidentalis* L. Allelopathic. Action of *C. occidentalis* can be substantiated due to soluble carbohydrates and amino acids present in the plant, which indirectly causes damage to the seed membranes of lettuce seed (Nayek, 2014). The membrane is the most significant site of a seed, which appears to be affected first by leachates having strong allelopathic action (Nayek, 2014). Anjum and Bajwa (2005), who studied the similar allelopathic effect of Cassia occidentalis, support the observations of the present study. Farooq et al. (2008) have also reported comparable outcomes during several allelopathic studies.

C. hirta is a noxious alien invasive weed species native to Central and South America, which has great potential to adversely alter natural mesic and hydric habitats and cause adverse impacts on cultivated lands on a large scale (Breaden, et al., 2012). The tested lettuce seedlings were affected by leaf litter of *C. hirata* that negatively affected the emergence of immature root and shoot upon its supplement. According to the studies done by Faravani et al. (2008), the inhibition of root and shoot growth were observed in the Barnyard grass seed which used the extracts isolated from shoot and root of *C. hirata*. The results of the present study also are in agreement with previous research findings as strong suppression of growth of lettuce seedlings were reported by leaf litter of *C. hirata* (Chon et al., 2002).

The tested crop species of the present study is lettuce (*Lactuca sativa* L.), an annual herbaceous plant of Asteraceae and interestingly, the weed species belong to the same family, family *Ageratum conyzoides, Sphagneticola trilobata, Chromolaena odorata, Crassocephalum crepidioides, Erigeron sumatrensis* and *Bidens pilosa* suppressed the growth of lettuce seedlings. That might be attributed by the interference of allelochemicals present in these species. Further findings of the present study are in agreement with the findings that ensured the allelopathic effects on plants that belong to the Asteraceae family (Inam et al., 1989). Moreover, it indicates that the strong growth inhibition on crop seeds might be the result of the plant species, which belongs to same plant family.

Results revealed that *Solanum americanum* of Solanaceae family and *Cuscuta reflexa* of the Convalvulaceae family exhibits statistically similar percentage of inhibitory behavior on growth of lettuce seedlings. *Cuscuta reflexa* is a common agricultural weed throughout the world, causing reduction of yields of many crops and if the infestation is heavy, causes the death of host. Kumari et al. (2017) also found the allelopathic potential of *C. reflexa* that caused an inhibitory effect on host plants of *Ziziphus mauritiana, Cajanus cajan and Ficus glomerata*. Further it was reported that 60-65% yield reductions in chillies, 31-34% in Greengram/ Blackgram, 60-65% in Niger, 87% in lentil, 86% in chickpea, 72% in tomato, and 60-70% in alfalfa due to the infestation *Cuscuta* (Mishra, 2009). *Solanum americanum* is one of the most widespread and morphologically variable species belongs to Solanum (Edmonds and Chweya, 1997).

In Fabaceae plants, allelopathy is regarded as a natural strategy that is shielding the plants against environmental enemies as well as competing with other plants (Xuan et al., 2006). According to the results of the present study, weeds have shown varying degrees of inhibitory behavior that ranges 45-81% within the species of Fabaceae family. This may be due to the different concentration of coumarins present in plant species of Fabaceae family which responsible for allelopathic behavior (Harborne, 1998).

The tubers of *C. rotundus* releasing harmful substances and allelopathic interactions play a key role in the defining the dissemination of plants in nature and yield of different crops (Xu et al., 2008). The observations made in the present study are not in close agreement with previous findings as leaf litter has been used in the present study and it might not be strong enough to express significant negative effect on seedling growth of lettuce and results were on par with the control treatment. Furthermore, *C. rotundus* contains a strong inhibitor of AChE in their tubers, which possibly acts as an agent to suppress other plants and become the worst invasive weed in the world (Sharma and Gupta, 2007). As leaf litter was only taken into an account in the present study, this might cause less allelopathy towards the germination of lettuce seedlings.

The lower level of allelopathic effects were reported in *Crassocephalum crepidioides* and *Phyllanthus debilis* that showed 34.6 and 36.4% of inhibitory percentages on growth of lettuce seedlings. Chen et al. (2009) stated that although *Crassocephalum crepidioides* has been listed as an invasive plant, it also cultivated as a vegetable in China, which indirectly indicated the less toxicity compared to other weed species. However, scientific evidence available on the allelopathic effect of *Phyllanthus debilis* is very limited in the literature. The Amaranthaceae family is well recognized for a smothering effect in vegetable fields and some *Amarathus* spp. are promising ground covers that suppress weeds in tropical countries (Fischer and Quijano, 1985). The higher inhibition percentage (65%) showed by *Amaranthus viridis* compared to other tested species of the Amaranthaceae family. Different species have shown varying degree of allelopathy ranging from 42.48% to 65%. Furthermore, *Cardiospermum halicacabum* showed similar results as *A. viridis* indicating 63% of inhibitory reaction.

Lantana camara and *Stachytarpheta urticaefolia* are notorious and invasive weeds and belong to the Verbenaceae family which retard germination, growth, development or metabolism of crops due to the discharge of allelochemicals (Qasem, 2001). *Ocimum sanctum* and *Hyptis suaveolens* are aromatic weeds, which are noxious, and world’s exotic persistent species invading the natural ecosystems (Rodrigues, et al., 2012). Results of the present study revealed a reduction in seedling height of lettuce under the allelochemical stress of both weed species, which is similar to the earlier reported allelopathic studies (Singh et al., 2013; Rodrigues, et al., 2012).

As per the results obtained in the present study, leaf litter of all twenty seven-weed species possesses allelopathic potential. The reduction in hypocotyl and radicle length might be attributed with allelochemicals by interfering major physiological processes of plant metabolism, *viz*. respiration and photosynthesis. Various inhibitors present in plants having allelopathic property reduce the overall metabolism of plants or plant parts, and predominantly anabolic activities are reported to be strongly decreased (Nayek, 2014). Therefore, use of green manure, compost or mulch using materials of weed species commonly found in croplands would be taken into serious consideration, as there might be growth retardation at any stage of plant growth particularly at initial stages such as seedling emergence. Furthermore, as allelochemicals are the secondary metabolites produced by plants, the understanding of plant interactions is important to enable the potential use of such allelochemicals by reducing dependency on herbicides in future cropping systems.

Conclusions

Leaf litter of most weed species considered in the present study showed adverse influence on lengths of hypocotyl and radicle and inhibitory percentage of lettuce seedlings under laboratory conditions. The growth of lettuce seedlings was strongly inhibited by plant species of *A. conyzoides, C. hirta* and *C. occidentalis.* Weed species belonging to the same family of lettuce also expressed a varying degree of inhibitory effects on growth performances of lettuce seedlings.

SUGGESTIONS

Further studies should be carried out to explore the mechanism of allelopathic activities of crop species as there is little or no information on laboratory and field studies. Purification of allelochemicals, identification of bioactive molecules and their effects should be investigated. Advance research is also needed to explore the allelopathic potential of the plant species in order to discover new molecular structures to be used in the control of invasive weeds and pests in agriculture systems, thus minimizing the damage caused by the hazardous synthetic agrochemicals. Moreover, there is a need for genetic and molecular studies of allelopathic plants, in order to investigate the bio-systemic pathways towards the defense against competitors, as well as to identify allelopathic genes for the transgenic improvement of economically important agricultural crops.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Multi-criteria decision system for greenhouse site selection ın Lower Euphrates Basin using geographic ınformation systems (GIS)

Burak Saltuk[1] and Ozan Artun[2*]

[1]Department of Biosystem Engineering, Agriculture Faculty, Siirt University, 56100 Siirt-Turkey.
[2]Karaisali Vocational School, Cukurova University, 01770 Adana-Turkey.

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Greenhouses have become quite a sought-after agricultural sector due to government support and private company preferences in recent years in Turkey. Greenhouse activities are only limited to Mediterranean and Aegean regions of Turkey and it is not much preferred in the inner regions. Due to these regions being preferred by people, mainly as tourism and settlement areas, it has become essential to find new areas for greenhouse operations. The climatic and topographical conditions in the Southeastern Anatolia Region, are appropriate regarding greenhouses. In this context, the Lower Euphrates Basin is selected as the study area. It covers four provinces (Adiyaman, Gaziantep, Kilis and Sanliurfa) and is one of the preferred regions for greenhouse operations in recent years. In this study, the climatic conditions and the production capacity of the Antalya province, which has the most greenhouse production areas of the Mediterranean Region and Turkey, are taken into consideration and the four provinces of the Lower Euphrates Basin were investigated for their compatibility with the desired conditions concerning green housing. Climate, soil, wind, altitude, slope, aspect, distance to rivers and lakes data are essential in choosing the greenhouse location. A Geographic Information System (GIS)-based multi-criteria evaluation for greenhouse site selection was applied in the study area by using these data. The validity of the results has been checked over with the field studies and also compared with the climatic conditions and production capacity of Antalya Province. The suitable, non-suitable or partially suitable areas for greenhouse siting in the basin were determined in terms of GIS, for guidance to producers and researchers in the future.

Key words: Multi-criteria decision analysis (MCDA), greenhouse siting, Lower Euphrates Basin, geographic ınformation system (GIS).

INTRODUCTION

Greenhouse production is one of the most important income generating branches of vegetable production. The primary need of the people for food is increasing with the growing population. Whether the production from agricultural areas is low or inadequate, the application of modern agricultural techniques can not meet the

*Corresponding author. E-mail: oartun@cu.edu.tr.

expectations from agriculture. Therefore, about 795 million people are undernourished globally, below 167 million over the last decade, and 216 million less than in 1990–1992. The decline is more pronounced in developing regions, despite significant population growth. In recent years, progress has been hindered by slower and less inclusive economic growth as well as political instability in some developing regions, such as Central Africa and western Asia (Anonymous, 2015). The increase in crop production is possible only if the production period is continuous. The greenhouse is the most crucial vegetable or fruit production material of our time. In the next century, the impact of the work will increase and this sector will become more interesting because of climatic changes or too much production demand. Greenhouse farming is a vegetable production plant where climatic conditions can be controlled and production control can be carried out. However, if done in climatic conditions that are not suitable, the economic benefit will bring damage instead.

In recent decades, greenhouse area has risen worldwide, due mainly to the increased use of plastic greenhouses for growing vegetable crops. Site selection is a critical factor for profitable and sustainable greenhouse production. The main factors determining the location and site selection of a greenhouse production area are: the cost of production, quality of produced yield, and transportation cost to markets (Nelson, 1985).

A wide variety of greenhouse technological packages are available nowadays, from simple plastic houses to very sophisticated glasshouses. Information on the growth strategies that can be afforded to conveniently supply the markets with high-quality vegetables year-round and in the greenhouse packages commonly used for that purpose are included, with particular reference to the Mediterranean Basin (Castilla and Hernandez, 2007).

Turkey's fastest-growing population, the reduction of agricultural areas due to climate change and the increase of residential areas have led people to find different production areas or to increase the number of products received from the unit area. The production can be continued for 12 months by increasing the greenhouse areas. Greenhouses are the vegetable production facilities, which enables production in the desired periods by keeping under control in indoor conditions such as temperature, humidity, CO_2, etc.

Since, air temperature and humidity are the two significant parameters affecting thermal comfort significantly, and only sensible load can be handled by an evaporative cooling system, the conventional evaporative cooling system is suitable for dry and temperate climate where the humidity is low (Costelloe and Finn, 2003; Heidarinejad et al., 2009).

During the production in greenhouses, internal environmental conditions need to be regular. Worldwide, studies have identified greenhouse and indoor conditions. The indicated species traditionally grown in the warm season, adapt to ambient temperatures ranging from 17 to 28°C and are sensitive to the cold. Temperatures persistently below 10 to 12°C over several days affect productivity, as do temperatures above 30 to 35°C. The daily variation between day and night average temperatures (thermal periodicity) is required for proper physiological functioning, with these thermal differences between 5 and 7°C. The minimum daily radiation requirements of these species are estimated at around 2.34 kWh m^{-2} day^{-1} which implies around 6 h of light per day, to a minimum total of 500 to 550 h of light during these three months. Other desirable climate parameters for these species would be the soil temperature of more than 14°C and ambient relative humidity of 70 to 90% (Nielsen et al., 1988).

Air temperature as well as solar radiation and air relative humidity is one of the most important variables of the greenhouse climate that can be controlled. It conditions not only crop development and production, but also energy requirements, which can account for up to 40% of the total production costs. The majority of plants grown in greenhouses are warm-season species, adapted to average temperatures in the range 17 to 27°C, with approximate lower and upper limits of 10 and 35°C. If the average minimum outside temperature is < 10°C, the greenhouse is likely to require heating, particularly at night. When the average maximum outside temperature is < 27°C, ventilation will prevent excessive internal temperatures during the day; however, if the average maximum temperature is > 27 to 28°C, artificial cooling may be necessary. The maximum greenhouse temperature should not exceed 30 to 35°C for prolonged periods (Kittas et al.,, 2013a).

In Turkey, there is no regular heating in the greenhouses. Generally, local heating is applied to protect the plants from frost. Failure to achieve the desired level of climate conditions and the lack of heating cause problems such as low yield, limitation in producing variety and the necessity of using hormones. The most considerable expense of the greenhouses is the electricity costs associated with heating and cooling. In the case of careful selection of the greenhouse site, it is thought that these costs can be considerably reduced and productivity can be increased.

In Mediterranean areas, less energy is used (500 to 1 600 MJ m^{-2}); however, heating is increasingly adopted to achieve early production and a constant quantitative qualitative yield, leading to higher energy use. Improved environmental control (e.g. more CO_2 supply, additional lighting), intensified production schemes and use of cooling systems all increase energy consumption. Average energy use accounts for 10 to 30% of total production costs, depending on the region (Kittas et al., 2013b).

Direction and speed of the wind are fundamental in the orientation of greenhouses. Covering the short axis of the greenhouse against the formation of natural ventilation

Figure 1. Map of Turkey and the study area (Saltuk et al., 2017).

Table 1. The data set used for MCDA modeling (Didan, 2015; Worldclim, 2018; GDRE, 2018; GTOPO30).

Factor Name	Description	Source
Wind	Wind direction and speed (m/s)	GDRE database
Land cover	Land Cover (-)	Derived from MODIS (MCD13Q1)
BIO5	Max temperature of the warmest month (°C)	www.worldclim.org/current
BIO6	Min temperature of coldest month (°C)	www.worldclim.org/current
BIO12	Annual precipitation (mm)	www.worldclim.org/current
Elevation	Altitude from the sea level (m)	Derived from GTOPO30
Slope	Slope in degrees obtained from altitude (%)	Derived from GTOPO30
Aspect	Aspect in degrees obtained from altitude (Direction)	Derived from GTOPO30
Dams	Distance to dams (m.)	Derived from Topographic map
Rivers	Distance to rivers (m.)	Derived from Topographic map
Soil	Major soil groups	MFAL database

facilities or winds at high speed will enable it serve for many years. Also, greenhouse positioning should be considered for maximum utilization of solar energy.

MATERIALS AND METHODS

Euphrates River is the longest river in southwest Asia; it is 2,800 km long and is one of the two main constituents of the Tigris-Euphrates River system. The river rises in Turkey and flows southeast across Syria and through Iraq (Anonymous, 2016). Four provinces in the basin of the Euphrates in Turkey (Adiyaman, Gaziantep, Kilis, and Sanliurfa) have formed the research area (Figure 1). In the study, elevation, slope, and aspect data was derived from GTOPO30 global elevation model with a resolution of 30 arc seconds (1 km) (GTOPO, 2018). Climatic data layers representing annual precipitation, maximum temperature of the warmest month, minimum temperature of the coldest month with a spatial resolution of about 1 km^2 were collected from Worldclim global database (Worldclim, 2018). Wind data were taken from the Turkey wind energy potential map prepared by the General Directorate of Renewable Energy (GDRE) and resampled to 1 km^2 resolution. The land cover coverage was taken from the Modis MODIS/Terra Vegetation Indices 16-Day L3 Global 250 m SIN Grid V006 (MCD13Q1) with 1 km resolution (Didan, 2015). Study area soil types are taken from general soil map of Turkey prepared by the Ministry of Food, Agriculture and Livestock (MFAL) (Table 1). Distance to dams and rivers were achieved by the evaluation of the topographic map of the study area in the GIS environment.

The criteria, which are essential in the selection of the greenhouse location, recommended in Table 2 were obtained from FAO (2017) publication and had been used in the study. Accordingly, the following method was used in the grading of the values. If the value is above the average value, it is used to make the classification by decreasing the value. In the study, the assigned values between 1 and 10 are determined, and the most effective one is given the value 10. For example, in the study, the maximum wind speed for greenhouses was evaluated by assigning 10 for the areas that have wind speeds less than 4.5 m/s. Areas with a wind speed of 9 m/s are given the value 2. The same method was used in the other criteria that were effective in choosing greenhouse locations.

After transferring all data to GIS environment, Multi-Criteria Decision Analysis method supported with Geographic Information Systems (GIS) was used for the suitability maps of the study area. ArcGIS 10.2 software was used for this purpose. In the MCDA method, often accepted factors in the literature and field studies that are effective in choosing the greenhouse construction site were appointed as impact factors and weight values (Table 2). 30 m × 30 m resolution factor value maps were created after assigning values to all the obtained factors, evaluated in the GIS environment.

Table 2. The factors affecting the greenhouse site selection (FAO, 2017).

Factor Name	Impact Class	Impact Factor	Weight value	Factor Name	Impact Class	Impact Factor	Weight value	Factor Name	Impact Class	Impact Factor	Weight value
	<4.5	10	10		0-100	1	7		18-22	10	7
Wind Speed (m/s)	4.5-5.5	9			500-600	8			22-26	9	
	5.5-6.5	8			600-700	10			26-30	8	
	6.5-7.5	5			700-800	10		**Max. Temperature of Warmest Month (^{o}C)**	30-34	5	
	7.5-8.5	3			800-900	9			34-38	4	
	8.5-10.00	2		**Annual Precipitation (mm.)**	900-1000	4			38-42	3	
	Brown Forest	8	5		1000-1100	5			42-46	2	
	Reddish Brown	10			1100-1200	6		**Min Temperature of coldest Month (^{o}C)**	-20 -16	1	10
Major Soil Groups	Limeless Brown	4			0-300	10	8		-16 -12	2	
	Limeless Brown Forest	5			300-600	9			-12 -8	3	
	Brown	8			600-900	5			-8 -4	4	
	Chestnut	2			900-1200	4			-4 0	5	
	Other	6			1200-1500	3			0- 4	7	
	Water	8	2		1500-1800	2			4-8	8	
	Evergreen Needleleaf forest	1			1800-2100	1			8-12	10	
				Altitude (m.)	2100-2400	1			0-1	10	6
	Evergreen Broadleaf forest	1			2400-12700	1		**Slope (^{0})**	1,01-8	6	
Land Cover	Deciduous Needleleaf forest	1			2700-3000	1			8,01-16	5	
	Deciduous Broadleaf forest	1			3000-3300	1			16,01-32	1	
	Mixed forest	1			3300-3600	1			32,01 - 75	1	
	Closed shrublands	1			3600-3900	1					
	Open shrublands	1		**Aspect**	Flat	7	8		>75	1	

Table 2. Contd.

Woody savannas	1	North	4	Distance to dams (m.)	< 500.	8	5
Savannas	1	Northeast	5		500 -1000	10	
Grasslands	4	East	7		1000- 5000	4	
Permanent wetlands	1	Southeast	10		>5000.	8	
Croplands	3	South	8	Distance to rivers (m.)	< 500	8	5
Urban and built-up	1	Southwest	10		500 -1000	10	
Cropland/Natural vegetation mosaic	1	West	7		1000-5000	6	
Snow and ice	10	Northwest	5		>5000	4	

The obtained raster maps were analyzed with the weighted sum function of the Spatial Analyst Tool in the ArcGIS 10.2 software. As a result of the analysis, suitability maps for greenhouse siting in the study area were obtained (Figure 2). The validity of this map was checked by the land studies and also a comparison of the greenhouse suitability map produced for Antalya with the same factor values.

In this study, we have tried to find a common intersection point by correlating the information with the topographical structure of the basin and combining this data with the long-term climatic data. We aimed to evaluate all the data together with the most suitable areas as possible and to generate maps of compliance in the numerical sense. From here it is aimed to implement both investors' knowledge about the application areas of the investors and to encourage the producers in the right areas as possible in the investments and incentives of the state.

RESULTS

In our study, we have investigated the suitability for greenhouse siting both in the four provinces of the Lower Euphrates Basin and in Antalya. Therefore, eleven criteria that could be effective in the construction of greenhouses were considered and the results compared to the province of Antalya, that is, one of the cities where greenhouse farming is mostly preferred in Turkey.

Because of the climatic conditions in the coastal areas of Antalya, the Serik district located to the east and the districts (Kumluca, Demre) in the south of the province has the most production potential. A large part of the total surface area of these districts is mainly covered with undergrowth production and citrus. Because the income of greenhouse cultivation is higher than conventional farming and it takes less time, citrus farming has shifted to the inner regions, and coastal regions have become areas where greenhouse cultivation is mostly applied. There are large-scale bazaars and commissions for vegetable growers in these territories. These three districts, mostly fulfill Turkey's potential for season vegetable farming. The fact that the total produced products reach the sellers and distribute them nationwide is like these commissions, and the producers sell all the goods in their hands, and producers are encouraging the establishment of new greenhouse areas. Antalya is surrounded by the western Taurus Mountains that limits the areas suitable for the greenhouse in the city. For this reason, it has been attempted to theoretically determine whether the study area will be an alternative to the Antalya province. The suitability values in MCDA calculations in the Antalya province were found in the range of 3.59 to 6.20. The same MCDA values are in the range of 5.16 to 6.20 and 4.69 to 6.20 for the coastal provinces (Kumluca, Demre) and Serik respectively. According to these values, the greenhouse existence of Antalya province also meets in these regions. Sanliurfa province is considered the closest province of Antalya concerning the appropriateness of greenhouse due to climatic and topographic conditions.

Therefore, regarding light transmission, it is recommended to build the greenhouse with an E–W orientation. Nevertheless, light uniformity is better in N–S greenhouses since the gutter and ridge shadows change their position during the

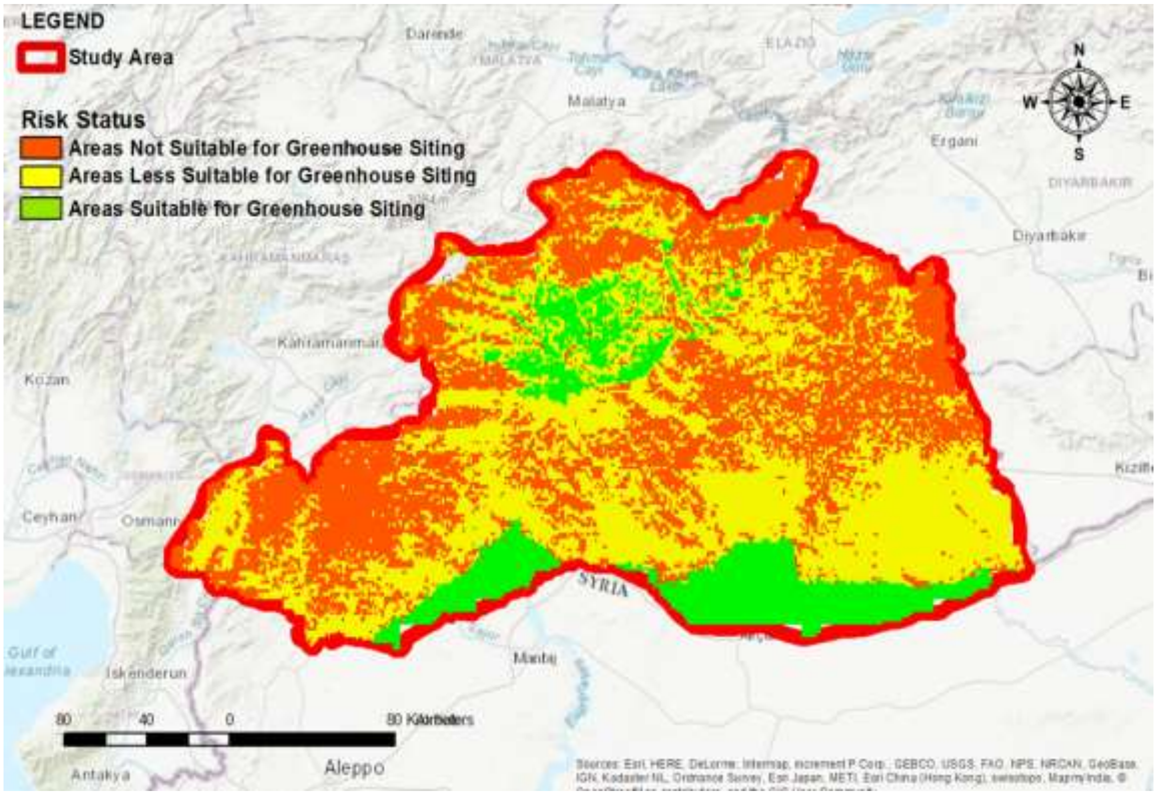

Figure 2. The suitability map of the study area.

day as the sun moves. In some Mediterranean areas, greenhouses are E–W oriented, but the crop rows are N–S for greater crop uniformity. About ventilation, it is advisable to build the roof ventilators perpendicular to the prevailing winds to enhance the air exchange (Montero, 2013).

The soil structure is significant for greenhouses. Although soilless farming options are used in some regions of our country to prevent the diseases caused by the soil and to form suitable growing areas, most of our producers still prefer grounded agriculture.

The nutrients dissolved in the soil's water are mainly nitrate, magnesium, potassium and sulfates. The quantity of these nutrients is ordinarily low and not sufficient for greenhouse crops which have high nutrient requirements (Sonneveld and Voogt, 2009).

The ideal EC is specific for each crop and dependent on environmental conditions (Sonneveld and Voogt, 2009); however, the EC values for hydroponic systems range from 1.5 to 2.5 ds m^{-1}. Higher EC hinders nutrient uptake by increasing osmotic pressure, whereas lower EC may severely affect plant health and yield (Samarakoon et al., 2006). The decrease in water uptake is strongly and linearly correlated to EC (Dalton et al., 1997).

Geographic information systems, which are now widely used in many areas, offer a wide variety of possibilities, especially in planning work. These systems which allows the collection, arrangement, questioning, and analysis of geographical data in a digital environment, accelerates geographically based works and to bring out the results with a more synthesist approach. It is quite easy to obtain the net result by comparison of the collected geographical data with the use of these systems in a short period (Bayar, 2005). The multifunctional applications of GIS make it possible to analyze spatial data, consolidate the information obtained from these analyzes, and store them. GIS helps to decide on the processing and distribution of agricultural land and can be used for administrative purposes (Sonmez et al., 2004).

Multi-criteria Decision Analysis (MCDA) is a solution applied in situations where more than one criteria must be evaluated together. The basic method of solving the problem is to divide the problem into small, understandable and straightforward pieces so that a meaningful result can be obtained from these pieces (Malczewski, 1999). On the other hand, MCDA provides a rich collection of techniques and procedures for structuring decision problems, along with designing, evaluating and prioritizing alternative decisions. At the most rudimentary level, GIS-MCDA can be thought of as a process that transforms and combines geographical data and value judgments (the preferences of the

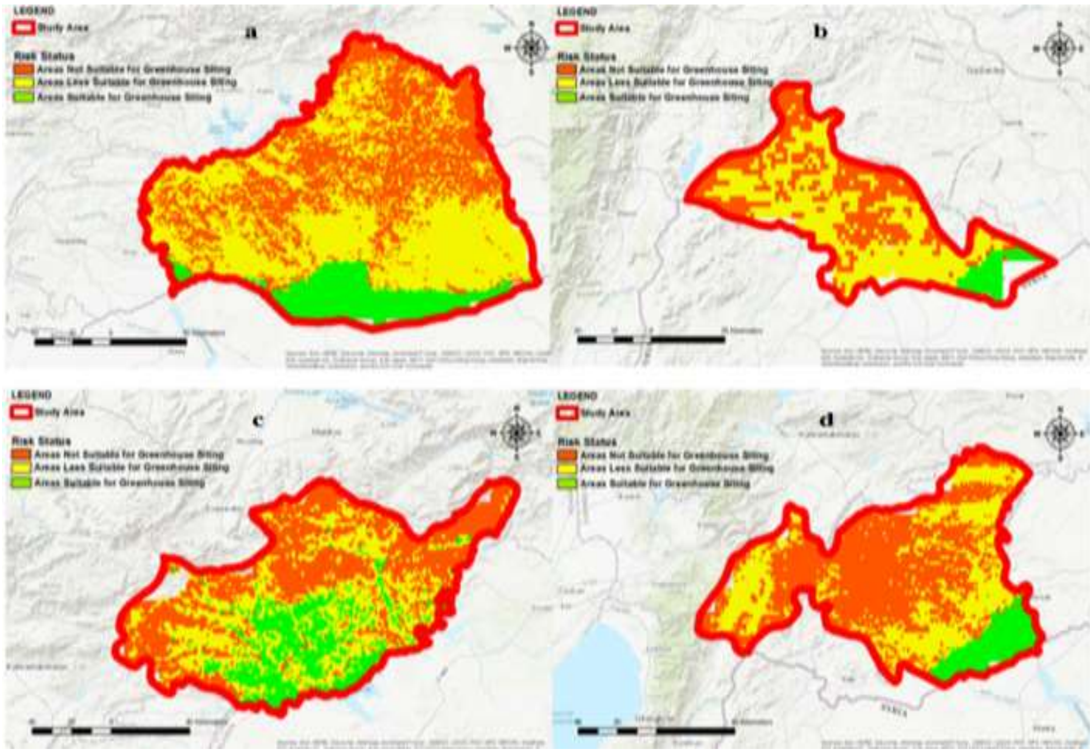

Figure 3. The Suitability Map of the provinces of the study area: (a) Sanliurfa province, (b) Kilis province, (c) Adiyaman province, (d) Gaziantep province.

decision-maker) to obtain information for decision making. It is in the context of the synergetic capabilities of GIS and MCDA that one can see the benefit of advancing theoretical and applied research on GIS-MCDA (Malczewski, 2006).

In the study, Geographic Information Systems (GIS) were used for analyses and querying, and Multi-Critical Decision Analysis (MCDA) was used for interpreting and solution. MCDA is a solution applied in situations where more than one criteria must be evaluated at the same time. It is known that, in the selection of the greenhouse location, if the climatic and topographic conditions are planned, there is a benefit in terms of stability and usability as well as economic benefit. Our study, especially encompasses Turkey's Lower Euphrates Basin in the Southeastern Anatolia Region, which is a potential area of greenhouse farm. It is aimed to determine the appropriateness of 4 provinces in the basin based on the province of Antalya and to determine the suitable locations for greenhouses in the basin. In this context, topographic characteristics (Elevation, slope, aspect), climate (maximum temperature of the warmest month, the minimum temperature of the coldest month and annual precipitation, wind direction and speed), land cover data and soil types of the study area have been used. This data is the primary material of the study.

The dataset mentioned above is layered in the ArcGIS environment, and Multi-criteria Decision Analysis is performed with this data. Besides, it is aimed to remind the producers of the importance of greenhouse farming in the region and to determine the greenhouse potential in the basin.

Sanliurfa province

When the study area is examined in terms of area size, the largest province in the study area is Sanliurfa. The total area of Sanliurfa province is 18584 km^2. Sanliurfa province has the third most farmland in Turkey after Ankara and Konya provinces. 64% of the province area is considered as agricultural land. In the study conducted, Sanliurfa center, southern and southeastern part of Urfa province has been determined to be suitable for greenhouse cultivation. It is seen that in Sanliurfa province, the districts of Viransehir, Ceylanpinar, Harran, and Akcakale consist of areas that are partially suitable or suitable for greenhouse farming. Nearly all lands of the Viransehir district can be defined as partially suitable and suitable areas for greenhouse farming. In our study, seven of the thirteen districts of Sanliurfa province were found partially suitable and suitable for greenhouse farming. In Sanliurfa, the percentage of areas suitable for greenhouse siting, partially suitable and unsuitable were determined as 12.03, 51.10 and 36.87%, respectively (Figure 3a).

Kilis province

In Kilis province, it is seen that the areas suitable for greenhouse construction were mostly collected in Elbeyli district and its vicinity. This district is followed by Kilis center, Polateli, and Musabeyli districts respectively. When the province of Kilis was examined, it was seen that the percentage of areas suitable for greenhouse siting, partially suitable and unsuitable were 7.21, 52.85 and 39.94%, respectively (Figure 3b).

Adiyaman province

In Adiyaman, the percentage of areas suitable for greenhouse siting, partially suitable and unsuitable were determined as 19.98, 37.24 and 42.78%, respectively. The central district and the eastern part of the province of Adıyaman consist of regions suitable for greenhouse cultivation. The most suitable regions for greenhouse cultivation are Samsat, Merkez and Besni districts. These districts are followed by Kahta district (Figure 3c). Currently, in Adıyaman province, 135000 m^2 greenhouse cultivation is performed and is mainly made of vegetables (tomato and cucumber). In Kahta district, benefiting from geothermal energy, production is carried out at 70000 m^2. Besides, there are also 65000 m^2 of soilless greenhouse cultivation areas. The hot water extracted from Turkey Petroleum Corporation’s (TPAO) oil wells in Kahta district, increase greenhouse investments in the region. The greenhouses heated by the hot water extracted from the oil wells in the Kahta district of Adıyaman provide employment and economic contribution.

Gaziantep province

Areas which are not suitable for greenhouse farming are located in the province of Gaziantep as a percentage (53.58%) because of its presence in the north and its climate. Only in the southern part of the province Gaziantep that some areas are identified suitable areas for greenhouses. When the Gaziantep province was examined, it was seen that Karkamis district is the most suitable district for greenhouse cultivation. This district is followed by Oguzeli and Nizip districts. Islahiye district town center and its surroundings also have suitable areas for greenhouse farming. In Gaziantep, the percentage of areas suitable for greenhouse siting, partially suitable and unsuitable were determined as 10.31, 36.11 and 53.58%, respectively (Figure 3d).

In the study area, the climate is temperate in these areas. It is topographically flat, 400-600 m above sea level, with the areas near the ponds or that are bordered by water sources and water collecting basins such as the Ataturk Dam. This allows the possibility of greenhouse cultivation in the early and late season for crop production. It is thought that this situation, which creates an important opportunity for vegetable production, especially for the Southeast Anatolian (GAP) Region market, can be supported by cheap and renewable energy sources such as biogas and geothermal, and this will further increase profitability.

DISCUSSION

In Turkey, greenhouse operations are limited only in the coastal areas of the Mediterranean and Aegean regions. It is not preferred in other regions. Nowadays, in the Mediterranean and Aegean regions, the suitable areas for greenhouse cultivation are preferred for tourism and residential areas. For this reason, it has become mandatory to find new areas for greenhouse enterprises. For this purpose, Southeastern Anatolia Region is considered suitable for the climate and topographic conditions. In this study, the criteria that are effective in the selection of greenhouses as a settlement are chosen from the literature.

Greenhouses need to be robust in terms of both static and strength. In particular, plant production structures such as greenhouse are produced from small diameter steel material due to the cost. Therefore, it cannot handle the wind load on it and demolitions occur. The air currents from the inner Anatolian wind pass to the Basra Bay and also show the research area as very risky in terms of wind load. In our study, the wind load coefficient of the long axis of the greenhouses that we sampled on the basis of provinces is higher than that of Antalya. Therefore, the wind factor was one of the most important factors in this study. Another important factor is the temperature. In particular, because the greenhouse indoor environment has only a layer of cover material thickness from the external environment, in terms of aquaculture, there has been a vast difference compared to Antalya. In the fall production period, 71% of the research area needs greenhouse heating.

The load combinations (wind, rain, and topography) of the effective loads in greenhouses were calculated both individually and together. All factors discussed in this study were classified according to their weight values, and the differences in Antalya were examined.

In the study area, the appropriate greenhouse siting areas were determined by MCDA analysis using the ArcMap 10.2 software. When the whole study area was examined, it was seen that suitable, less suitable and inappropriate areas for greenhouse siting are 13.23, 45.38, and 41.39%, respectively. Upon examining the provinces in the study area, Adiyaman (19.38%) and Sanliurfa (12.03%) provinces are those with the highest percentage of suitable areas for greenhouse siting in the basin. When the study area was examined on the basis of districts, it was seen that the most suitable areas for greenhouses are in Viransehir and Elbeyi districts. As a result, the use of this work in the greenhouse activities to be made in the Lower Euphrates Basin region in the coming years may help farmers use the resources properly. The Sanliurfa-Karaali region has a potential of the geothermal greenhouse, and the heating costs of this region can be considered an advantage. As a result of the questionnaires and evaluations made by public companies and private companies in the study area, it is stated that greenhouse activities should be among the priority policy areas. 73% of representatives of public institutions, 67% of NGO representatives and 63% of university representatives stated that greenhouse should be among the priority policy areas (Anonymous, 2014).

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Agromorphological traits variation in local sorghum varieties from the North region of Burkina Faso and identification of some interest traits

Clarisse Pulchérie Kondombo[1*], Issa Karambiri[2], Adama Kaboré[1], Schémaéza Bonzi[2] and Irénée Somda[2]

[1]Institut de l'Environnement et de Recherches Agricoles (INERA), BP 10 Koudougou, Burkina Faso.
[2]Université Nazi Boni (UPB), BP 1091 Bobo-dioulasso, Burkina Faso.

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The aim of the study was to assess the agromorphological diversity of 150 local sorghum varieties collected from 13 villages of the North region of Burkina Faso, in order to understand the dynamics of diversity and to identify interest gene pools for varietal improvement. The varieties were characterized from 2017 to 2018 at INERA Saria Research Station in the North-Sudanian zone (700-900 mm of rainfall). The experimental design was an alpha lattice with three repetitions. Twenty-four qualitative and quantitative traits were used to assess the local sorghum. Seven qualitative traits of the panicle were allowed to identify four main botanical races and two intermediate races: guinea (86.0%), caudatum (6.7%), bicolor (0.7%), durra (0.7%), guinea-caudatum (2.0%) and durra-bicolor (4.0%). The anthocyanin varieties were predominant (99.3%) like those with white pericarp (62.7%). The highest variances were observed with cycle length and the weight of 100 grains as well between varieties as in each village; these two traits were the most heritable (h^2 = 90). The local sorghum varieties were structured into three groups by hierarchical cluster analysis on the basis of cycle length, grain weight, stem height, leaf length and 100-grains weight. The group of short cycle varieties (69 days) with short stem height and better 100-grains weight is the most productive. Therefore, this group is particularly interesting for varietal improvement.

Key words: Sorghum, local varieties, agromorphological variation, botanical race, cycle, heritability.

INTRODUCTION

Sorghum [*Sorghum bicolor* (L.) Moench] is the fifth major cereal grown in the world after corn, rice, wheat and barley. On average, it takes up 42.8 million hectares with a grain production of 63.4 million tons (FAOSTAT, 2012-2016). The major part of cultivated areas (64.3%) is found in Africa, whereas the grain production represents only 42.2% of the world production. The average grain yield for Africa is low (970 kg/ha) compared with that of the other countries in the world (2399 kg/ha). Such differences in yield between Africa and the rest of the

*Corresponding author. E-mail: clarissebk@yahoo.fr.

world are due to the technical and economic means used in the production. Bindraban et al. (2000) and Leiser et al. (2014) mentioned that the major constraints of sorghum production in Africa are the water availability and the deficiency of soil mineral nutrients, particularly in phosphorus. Ayana and Bekele (1998), Mujaju and Chakauya (2008), Ngugi and Maswili (2010), Dossou et al. (2015), Desmae et al. (2016) and Teshome et al. (2018) observed that the sorghum growing in Africa was still widely based on local varieties that remain an important component of traditional farming systems.

In Burkina Faso, sorghum is the first food crop, followed by millet, corn, rice and fonio. On average it takes up to 42.6% (1.7 million hectares) of cereal growing area with a grain production of 37.5% (1.7 million tons), (MARHASA, 2012-2016). The farming systems of sorghum are still extensive due to the low investment capacity of farmers (low level of agricultural equipment, the non-use or low use of mineral fertilizers, etc.); moreover, sorghum is often cultivated on marginal soils.

Studies have shown that local sorghum varieties were the most widely cultivated in Burkina Faso (Zongo, 1991; Barro-Kondombo et al., 2010; Nebié et al., 2009; Sawadogo et al., 2014). These local varieties mostly (93 to 96%) belong to the guinea botanical race (Harlan and De Wet, 1972). The varieties of this race (although their low agronomic performance) are preferred by farmers for their rusticity, their sensitiveness to photoperiod which better suit to the production conditions of traditional farming systems; their high stem and grain quality are preferred respectively for various domestic and culinary uses. Some improved sorghum varieties of caudatum botanical race have been developed and released by sorghum research program; despite their higher productivity, they are much less cultivated due to their weak adaptability to the extensive systems production.

The constraints of sorghum growing have always existed in Burkina Faso, but their nature and importance vary according to farming areas. In the North region, sorghum takes up on average 49.6% of cereal growing areas before millet (46.1%). The low agricultural potential of cultivated land (DGAT, 2006), shortening of the rainy season and the bad distribution of rainfall (Paturel et al., 2010), as well as bioagressors attacks, particularly striga (*Striga hermonthica*) are the major constraints which affect sorghum yields in the region. Some old low performing varieties have been given up and many new varieties were introduced in the villages by farmers (Kondombo et al., 2016). Thus, a collection of local sorghum varieties was carried out in 2009 in the four provinces of the region (Zondoma, Passoré, Yatenga and Lorum) and seed were stored in the gene bank of INERA Saria Research Station in Burkina Faso. Owing to the local differences between the villages and the interactions between genotypes and environments, the local sorghum varieties cultivated by farmers in the North region could show significant and useful biological traits.

This study was undertaken to assess the agromorphological diversity of local sorghum varieties from 13 villages of the North region of Burkina Faso to determine the structuration of the diversity and identify gene pools that could be of immediate use for the sorghum varietal improvement.

MATERIALS AND METHODS

Collection site

The plant material of this study was collected from 13 villages of the four provinces in the North region of Burkina Faso (Zondoma, Passoré, Yatenga and Lorum) according to the method described by Kondombo et al. (2016). The villages are situated between latitudes 12°38' and 14°18' north and between 1°55 and 2°95 longitudes west (Figure 1). The climate of the region is a Sudano-Sahelian type, with a rainfall of 500 to 700 mm. The prevailing types of soil are Ferric-lixisol (39.4%) and lithosols on hardpan with gravelus (33.3%). The average dates of the beginning and the end of rainy season in the region are around 28th June and 21st September (National Direction of Meteorology, 2011).

Plant material

The characterization of agromorphological diversity was carried out with 150 local sorghum varieties from 2017 to 2018 at INERA Saria Research Station, situated in the North-Sudanian zone, at 12°16' North latitude and 2°09' West longitude, at 300 m altitude. The list of sampled villages is reported in the Table 1.

METHODOLOGY

Experimental design and crop management

The experimental design was an alpha lattice with three repetitions. Each repetition includes 15 blocks of 10 varieties. The sowing was carried out on 12th July, 2017. By repetition, each variety was sown on one row of 6 m length with spaces of 80 cm between rows and 20 cm between seed holes. The thinning was carried out 14 days after sowing, leaving one plant per seed hole. Mineral fertilizers NPK (14N-23P-14K-6S-1B) were applied at 100 kg/ha and urea at 50 kg/ha (46% of nitrogen) respectively 14 and 40 days after sowing. The utile rainfall for the trial was 444.2 mm.

Assessed traits

Thirteen qualitative traits and eleven quantitative traits were used to describe sampled varieties (Tables 2a and 2b, respectively). Apart from the number of leaves recorded once a week from the thinning up to the appearance of the last leaf, the other traits were observed or measured at the stage of heading, milky grain stage, physiological maturity and after harvests (Table 2).

Data analysis

A Multiple Correspondence Analysis (MCA) was carried out with the average values of seven qualitative traits describing the panicle. Twenty five modalities of these traits were used in order to establish the structuration of the types of panicle and to identify the botanical races of the study according to the classification of Harlan and De

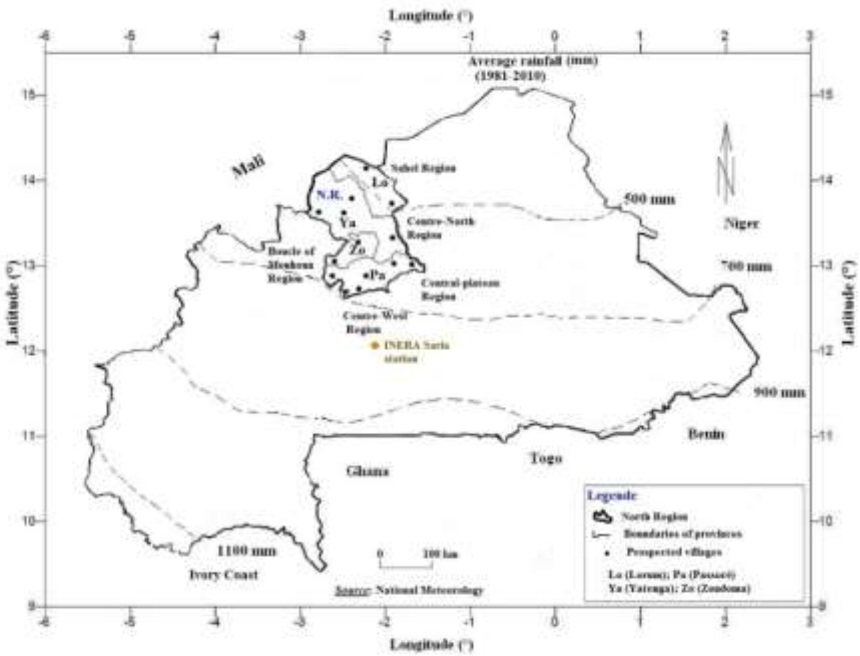

Figure 1. Geographic location of the surveyed villages in the North region of Burkina Faso (source: Ministry of Territorial Administration, 2010; National Direction of Meteorology, 2011). Lo (Lorum), Pa (Passoré), Ya (Yatenga), Zo (Zondoma).

Wet (1972). These traits are: the panicle compactness (PAC), the spikelet pedicellate persistence (SPP), the glumes length (GLL), the glumes opening (GLO), the grain rotation (GRR), the grain form (GRF) and the glumes adherence (GLA).

Regarding the quantitative traits, the variances analysis (ANOVA) were carried out at two levels to evaluate variety effect and the village effect on traits expression according to the following models: $Y_{ijk} = \mu + \tau_i + \gamma_j + \rho_{jk} + \varepsilon_{ijk}$ for the variety and $Y_{ij} = \mu + \alpha_i + \gamma_j + \varepsilon_{ij.}$ for the village; μ is general mean of the measured trait, τ_i mean of the variety i, γ_j effect of the repetition j, ρ_{jk} effect of block k in the repetition j, ε_{ijk} residual effect for variety; α_i variety effect within the village and ε_{ij} residual effect for village. The broad sense (H^2) and the narrow sense (h^2) heritabilities were calculated for each trait.

The structuration of agromorphological diversity was established by a Hierarchical Cluster Analysis (HCA) on the basis of the Euclidean distance according to Ward's aggregation criterion. The groups from the HCA were characterized by a Factorial Discriminant Analysis (FDA) in order to identify the most discriminating traits of the groups. The FDA was used to check if the varieties were different from one village to another.

The ANOVA was performed using GenStat software version 14.2, whereas the MCA, HCA and FDA were carried out with the XLSTAT software version 2018.2 (Addinsoft, 2018).

RESULTS

Evaluation of qualitative traits

Almost all the 150 varieties assessed had leaf anthocyanin pigmentation (99.3%). The varieties with white leaf midrib (94.7%) and black glumes (77.3%) were the most common. The most differentiated modalities were observed on the pericarp colour (six modalities): the white pericarp was predominant (62.7%), followed by red pericarp (18.7%), orange (7.3%), brown (4.0%), grey (2.7%) and yellow (1.3%); while 3.3% were mixtures. Seventy-eight percent of the varieties had grains without testa and 64.7% had presented good corneous to medium endosperm texture.

The modalities of the seven qualitative traits of the panicle have allowed identification for four main botanical races of sorghum and two intermediate races. The main races were: the guinea which represent 86.0% [two sub races: the gambicum (96.1%) and the margaritiferum (3.9%)], the caudatum (6.7%), the bicolor (0.7%) and the durra (0.7%). The intermediate races were: the guinea-caudatum (2.0%) and the durra-bicolor (4.0%). The structuration of the modalities of panicle traits is shown in the Figure 2, whereas the botanical races identified in the study is presented in the Figure 3.

Evaluation of quantitative traits

Effect of variety on the traits expression

The ANOVA (Table 3) showed very highly significant differences ($P < \alpha = 0.001$) between the 150 local sorghum varieties for all the eleven quantitative assessed traits. The most important variations was observed with

Table 1. Geographic origin of the 150 local sorghum varieties assessed.

Province	Isohyet (mm)	Department	Village	Number of varieties per village
Loroum	400-500	Sollé	Sollé	7
		Ouindigui	Ouindigui	6
Yatenga	500-600	Koumbri	Pogoro Mossi	14
		Ouahigouya	Gourga	11
		Seguenega	Zomkalaga	7
		Tangaye	Tougué Mossi	10
Zondoma	500-600	Boussou	Toubyengo	13
		Léba	Léba	11
Passoré	600-700	Gomponsom	Zougoungou	17
		Kirsi	Koussaogo	12
		Latodin	Sissamba	14
		Pilmpikou	Sandia	15
		Yako	Douré	13
Total		13	13	150

Table 2. List of qualitative and quantitative traits.

Name of trait	Abbreviation	Modality
Leaf pigment colour	LPC	Tan (1), anthocyanin (2)
Leaf midrib colour	LMC	White (1), green (2), yellow (3), brown (4), mixed (5)
Glumes colour	GLC	Yellow (1), brown (2), red (3), black (4), mixed (5)
Pericarp colour	PEC	White (1), yellow (2), orange (3), grey (4), brown (5), red (6), mixed (7)
Seed subcoat	SSC	Present (1), absent (2), mixed (3)
Endosperm texture	ENT	1 to 5: 1 (completely corneous), 5 (completely starchy)
Panicle compactness	PAC	Very loose (1) loose (2), semi-loose (3), semi-compact (4), compact (5), very compact (6)
Spikelet pedicellate persistence	SPP	Absent (1), low (2), medium (3) persistent (4)
Glumes length	GLL	Shorter (1), equal (2), longer than grain (3)
Glumes opening	GLO	Completely closed (1), weak (2), medium (3), very open (4)
Grain rotation	GRR	Absent (1), weak (2), medium (3), full rotation (4)
Grain form	GRF	Globulous (1), elliptic(2)
Glume adherence	GLA	Absent (1), weak (2), medium (3), strong (4)
Cycle duration from sowing to 50% heading (days)	NDH	Whole plot
Leaves number	LVN	3 randomly main stem marked from the 5-6 leaves stage to the flag leaf
Plant height (cm)	PLH	3 main stem recorded from the base of the stalk to the tip of the panicle (cm)
Stem diameter (mm)	STD	3 main stem recorded at the third inter-node under panicle
Length of third leaf under panicle (cm)	LTL	3 main stem
Width of third leaf under panicle (cm)	WTL	3 main stem
Panicle length (cm)	PAL	3 main stem
Sugar content (% brix)	SUC	1 main stem using a refractometer
Panicle dry weight (g)	PAW	12 hills
Grain dry weight (g)	GRW	12 hills
100 grains weight (g)	1GW	Bulk of 12 hills

the cycle duration from sowing to 50% heading (NDH) and the average weight of 100 grains (1GW) with 28.2 and 27.9 values of F, respectively. The NHD varied from 54 to 82 days and the weight of 100 grains from 1.2 to

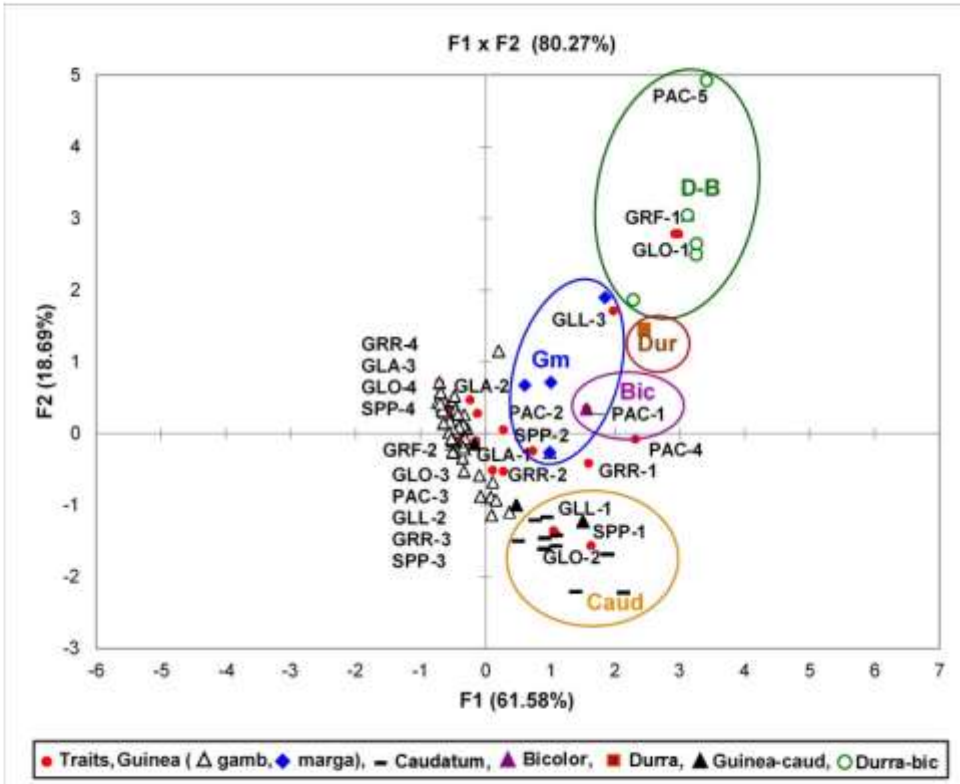

Figure 2. Racial structuration of the 150 local sorghum varieties of the North region of Burkina Faso determined by Multiple Correspondence Analysis (MCA) on the basis of seven qualitative traits.

4.1 g. The varieties with red pericarp were on average earlier cycle (67.4 days) compared to the varieties with white pericarp (72.1 days); their average weights of 100 grains were 2.3 g and 2.1 g, respectively. The earliest variety was Kapambga (Gg) (54 days) and the latest were Bobdo (Gg), Bininmênêm (Gg) and Kendezouanga (Db), who presented all 82 days of cycle duration (NDH). For the weight of 100 grains, Zonombdo (guinea-margaritiferum) (1.2 g) and Loumba (durra) (4.1 g) varieties had respectively the extreme values.

The broad sense heritability (H^2), including genetic effects as well as environmental effects varied from 0.55 to 0.97. It was particularly higher for the cycle duration (0.97) and the weight of 100 grains (0.96); as the number of leaves and length of leaves both showed a H^2 of 0.91. The narrow sense heritability (h^2) was higher for the cycle duration (0.90), the weight of 100 grains (0.90), the length of leaf (0.77), the number of leaves (0.76) and the Brix (0.66). The lowest heritability was observed for weight of panicles (0.28) followed by dry grains weight (0.32).

Effect of village on the traits expression

At the village level, the ANOVA revealed significant varietal differences in each village for cycle duration, the weight of 100 grains, the number of leaves and the length of leaves. The least differentiated traits in the most villages were the stem diameter, the leaf width, the panicles weight and the grains weight. The variability of varietal traits was more important within the varieties of the villages of Sissamba, Koussaogo, Léba, Porgo-mossi and Toubyango (Table 4).

Between villages, the most significant differences of traits were cycle duration and leaves length. The Lambda of Wilks test from Factorial Discriminant Analysis (FDA) gave a probability of 0.015 showing that the varieties are significantly different from one village to another. The differentiation was more important between the varieties of the village of Zougoungou and those of the villages of Gourga and Sollé; also, between the varieties of the village of Sollé and those of the villages of Douré, Koussaogo, Léba, Sandia, Sissamba and Zomkalaga.

Structuration of the diversity

The hierarchical cluster Analysis (HCA) discriminated the 150 local sorghum varieties into three groups (Figure 4). These are: i) Group 1 contains 58.7% of the varieties

Figure 3. Main botanical races identified in the 150 local sorghum varieties assessed.

from all the 13 villages. It has the earliest and most productive varieties, with a shorter plant height and a better weight of 100 grains; ii) Group 2 contains 30.7% of the varieties from 12 villages except the village Zomkalaga. This group includes varieties whose traits are intermediate between the first and third groups; iii) Group 3 includes 10.6% of the varieties from seven villages which are Sandia, Douré, Zougoungou, Sissamba, Tougué-mossi, Zomkalaga and Toubyango. This group is constituted by late maturing varieties characterized by lower productivity, taller plants and an average lower weight of 100 grains. The guinea-margaritiferum varieties are grouped into Group 1, while red pericarp varieties are classified into Groups 1 and 2. The description of the three groups is given in Table 5. The groups were discriminated in order by the cycle duration from sowing to heading, the weight of grain, the plant height, the leaves length and the average weight of 100 grains, with a probability $p < 0.0001$. The values of the proximity matrix established on the basis of the Euclidian distance showed that the 150 local sorghum varieties even those identified under the same names were all different from each another (sometimes weakly) for all eleven assessed traits.

DISCUSSION

Diversity of botanical races and variability of qualitative traits

Analysis of the seven descriptive traits of the panicle showed a predominance of the guinea botanical race varieties (86.0%) in the North region of Burkina Faso. This racial preference by farmers could be explained by four main reasons which are: i) the history of the domestication of botanical sorghum races, ii) the cultural heritage in terms of varieties, carried on from generation to generation by the local populations, iii) the adaptability of guinea to the extensive farming systems as practiced by most farmers in Burkina Faso, iv) their sensitivity to short days photoperiod and their good grain quality. The cultivation of the other races [caudatum (6.7%), durra (0.7%), bicolor (0.7%)] would meet the needs of specific uses. The caudatum varieties would serve as lain period sorghum, particularly those with sweet grain are consumed fresh before the harvests. The durra variety identified in this study is weakly used in human diet, but can be consumed in the form of porridge or mixed with cowpea in cooking; it would be used more specifically in traditional medicine for the treatment of goiter "hypothyroidism". The bicolor variety found in this study is called "Kankan-siido" in the region local language, and is cultivated for its sweet stem.

The racial composition of the local sorghum varieties of this study is similar to that reported by Zongo (1991) in Burkina Faso. However, the frequency of the guinea varieties is lower than those reported by Zongo (1991) and Barro-Kondombo et al. (2010) who found 93.1% across Burkina Faso and 94.4% at the level of three regions (Centre-North, Centre-West and the Boucle of Mouhoun), respectively.

The great presence of the anthocyanin varieties (99.3%) is noticeable in the local sorghum varieties. The reasons why farmers cultivate those varieties were not provided at the time of varieties collection. This strong

Table 3. Statistical parameters and heritabilities of the eleven quantitative traits of the 150 assessed varieties.

Source of variation	NDH (days)	PLH (cm)	STD (mm)	LVN	LTL (cm)	WTL (cm)	PAL (cm)	PAW (g)	GRW (g)	1GW (g)	SUC (% of brix)
Minimum	54.0	168.3	9.7	17.7	54.0	5.7	20.0	193.6	137.3	1.2	6.2
Maximum	82.0	435.0	15.0	25.0	85.3	10.7	43.7	811.8	641.6	4.1	18.6
Mean ± SD	71.0 ± 5.4	284.0 ± 48.3	12.4 ± 1.0	21.4 ± 1.3	68.0 ± 5.7	7.9 ± 0.8	30.3 ± 4.5	481.0 ± 137.0	349.0 ± 107.0	2.2 ± 0.4	12.6 ± 2.1
F repetition	0.4 ns	5.1*	4.2*	1.8 ns	4.5*	5.3*	6.1*	5.4*	5.2*	3.1*	0.6 ns
F block	15.2***	8.2***	3.1***	5.5***	4.3***	2.6***	3.5***	4.5***	4.6***	13.1***	3.6***
F variety	28.2***	5.0***	2.4***	10.6***	11.3***	3.7***	2.8***	2.2***	2.4***	27.9***	6.8***
CV (%)	2.3	9.8	6.2	2.8	3.9	7.1	10.9	21.4	22.5	5.2	9.4
H^2	0.97	0.80	0.58	0.91	0.91	0.73	0.65	0.55	0.60	0.96	0.85
h^2	0.90	0.57	0.31	0.76	0.77	0.48	0.38	0.28	0.32	0.90	0.66

ns (non significant)
* (significant effect of the factor at α = 0.05 level),
** (highly significant effect of the factor at α = 0.01),
*** (very highly significant effect of the factor at the α = 0.001).

Table 4. Results of ANOVA of the variety effect by village and between villages.

Villages	NDH (days)		PLH (cm)		STD (mm)		LVN		LTL (cm)		WTL (cm)		PAL (cm)		PAW (g)		GRW (g)		1GW (g)		SUC (% brix)	
	μ	F	μ	F	μ	F	μ	F	μ	F	μ	F	μ	F	μ	F	μ	F	μ	F	μ	F
Douré	72.6	22.6***	290	4.7***	12.3	0.9 ns	21.1	18.4***	67.2	11.2***	7.7	4.0**	29.3	1.4 ns	428	1.4 ns	297	1.5 ns	2.1	36.5***	13.2	2.0 ns
Gourga	69.2	35.4***	284	1.9 ns	12.9	2.4*	21.0	6.5***	72.0	5.8***	7.8	2.1 ns	32.0	2.7*	538	1.7 ns	382	1.7 ns	2.2	36.8***	12.4	2.1 ns
Koussaogo	70.6	17.8***	290	1.8 ns	12.3	3.4**	21.1	6.9***	68.7	6.2***	8.0	1.7 ns	31.0	3.3**	484	2.4*	360	2.4*	2.2	18.2***	12.1	2.8*
Léba	71.3	30.8***	270	5.4***	12.6	1.5 ns	21.2	21.8***	66.6	19.7***	8.2	3.4**	29.3	7.9***	481	2.3 ns	376	3.0*	2.4	43.0***	12.6	3.6**
Ouindigui	69.8	29.0***	271	2.6 ns	12.3	0.8 ns	21.3	14.8***	68.2	31.1***	7.8	1.9 ns	31.6	2.7 ns	547	5.7**	383	4.0*	2.3	51.6***	13.8	8.8**
Porgo-mossi	71.1	27.5***	277	3.9**	12.4	1.3 ns	21.3	17.7***	68.4	16.0***	7.8	2.1*	31.5	2.9*	461	2.5*	334	2.1 ns	2.1	49.4***	12.4	2.5*
Sandia	72.8	15.6***	296	1.7 ns	12.2	1.6 ns	21.8	14.5***	66.7	7.0***	7.8	1.2 ns	30.0	2.7*	477	1.1 ns	352	1.7 ns	2.2	19.1***	12.6	1.2 ns
Sissamba	72.4	23.8***	290	3.9**	12.4	3.2**	21.4	16.4***	68.0	9.6***	7.9	3.9**	30.0	2.9*	460	4.2***	340	3.7**	2.2	22.6***	11.7	1.9 ns
Sollé	69.5	33.3***	271	1.4 ns	12.8	0.4 ns	21.2	7.5**	72.6	11.4***	8.2	1.2 ns	32.3	2.1 ns	502	2.5 ns	333	4.7*	2.1	10.4***	12.8	6.3**
Toubyango	67.9	16.7***	273	8.3***	12.9	2.9*	21.3	5.7***	68.1	7.9***	8.1	5.6***	29.4	4.0**	504	2.0 ns	370	1.9 ns	2.1	37.4***	12.8	8.2***
Tougé-mossi	70.3	25.5***	278	2.6*	12.3	5.0**	21.2	8.1***	67.9	4.2**	7.5	0.9 ns	29.8	0.5 ns	465	1.7 ns	318	2.4 ns	2.2	8.4***	13.1	1.7 ns
Zomkalaka	73.1	25.1***	301	3.3*	12.5	2.2 ns	21.9	20.3***	67.9	3.2*	8.1	2.2 ns	30.1	4.3*	476	0.9 ns	354	1.2 ns	2.3	25.8***	12.2	4.1*
Zougougou	71.8	18.4***	287	6.6***	12.1	1.8 ns	21.7	2.9**	65.3	6.8***	7.8	1.9 ns	29.9	2.2*	484	1.9 ns	356	2.4*	2.2	17.4***	12.2	3.7***
Inter-villages		3.1***		1.4 ns		2.7**		1.8*		4.3***		2.1*		1.7 ns		1.7 ns		2.0*		1.6 ns		1.5 ns

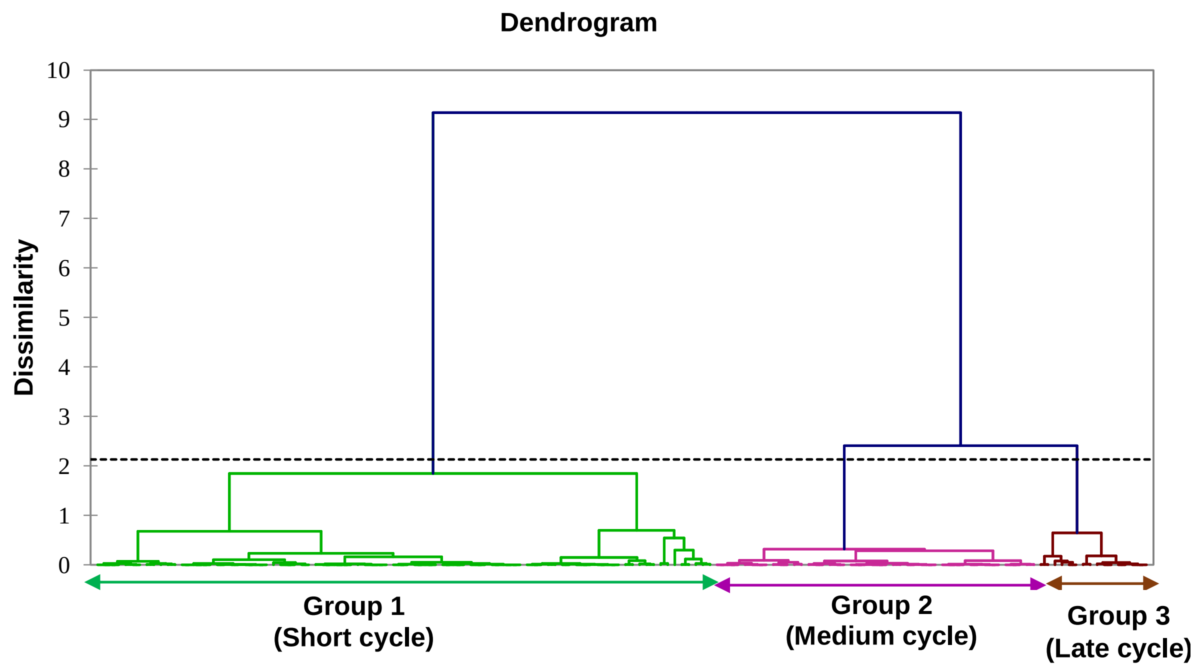

Figure 4. Agromorphological structuration of the 150 local sorghum varieties determined by HCA according to Ward's aggregation criterion.

Table 5. Description of the three groups of HCA with some discriminating traits.

HCA groups	NDH (days)	GRW (g)	PLH (cm)	LTL (cm)	1GW (g)
Group 1	69.2	388.5	276.8	68.9	2.3
Group 2	72.6	305.7	285.7	67.5	2.1
Group 3	76.6	258.8	316.3	64.4	2.0

adoption of the anthocyanin varieties would not be by fortuitous, but probably linked to the biotic and abiotic stresses. In fact, Etasse (1977) has found that the sorghums without anthocyanin would be more susceptible to foliar diseases especially to *Ramulispora sorghi*. Dicko et al. (2005) have emphasized that the sorghum varieties with high proanthocyanidins content showed better resistance to pre and post flowering drought. The importance of the phenolic compounds of sorghum has been reported by several authors who revealed that anthocyanins and other flavonoids have medicinal properties: antioxidants (Awika and Rooney, 2004), anti-inflammatory (Burdette et al., 2010) and anti-cancer (Wu et al., 2011). The presence of tannins in the grain would reduce the digestibility and fight obesity (Awika and Rooney, 2004; Wu et al., 2012).

The results of this study are similar to those reported by Barro-Kondombo et al. (2010) who found 98.4% of anthocyanin sorghum varieties. Multi-scale investigations could provide more information on the nature of phenolic compounds of the local sorghum varieties in Burkina Faso and the reasons why farmers prefer this trait. The results also showed a great presence of white pericarp varieties. Generally in Burkina Faso, white pericarp varieties are used in cooking to prepare thick porridge, a local dish widely consumed in rural families. On the other hand, red pericarp varieties are used in the preparation of local beer sold or served during traditional ceremonies. Some red pericarp varieties have a sweet grain and are sown to be consumed fresh during the lain period, as the earliest variety named Kapambga "sorghum whose grain is extracted fresh from the panicle".

Diversity and structuration of measured traits

The inter-varietal effect was very highly significant for all the eleven assessed traits, showing that the varietal diversity cultivated by the farmers was high. The greatest variations was observed with the cycle duration from sowing to heading and the average weight of 100 grains; these two traits strongly contribute to the differentiation of the local sorghum varieties assessed. At the village level in addition to the cycle duration and the weight of 100 grains, the number of leaves and the length of leaves were the traits which contributed to the varietal differentiation. The variability of the cycle within varieties was an expected result. In fact, the North region of Burkina Faso is an area more exposed to rainfall fluctuations. The beginning and the end of the rainy season is always uncertain, for that reason farmers integrate possible alternatives into their production strategies to maximize the chances of harvest. In case of an early beginning of rains the later-cycle varieties are the first to be sown and in the otherwise, short-cycle varieties are sown. Only farmers having farming areas around lowlands manage to sow late maturing sorghum varieties. Among the factors influencing crop diversity, climate effects are the most important Mercer and Perales (2010). Vom Brocke et al. (2010) also reported that the adaptability of the cycle was one of the criteria in sorghum growing areas in Burkina Faso.

The great variability of the weight of 100 grains is in relation to the various food uses. The guinea margaritiferum varieties, which have the smallest grains (1.2 g), dehulled and used alternative of rice. The sorghums with medium grain are used for the preparation of common dishes like “tôt”, local beer whereas the sorghums with big grain like the durra (4.1 g) are used in the preparation of porridges and couscous.

In term of traits heritability, our results are quite similar to those obtained by Tamini (2014) in a study on improved sorghum varieties of Burkina Faso. Tamini (2014) reported slightly higher values of heritability on the cycle ($h^2 = 0.98$), the weight of 1000 grains ($h^2 = 0.85$), the length of leaves ($h^2 = 0.81$) and the number of leaves ($h^2 = 0.94$). Some traits may not be selected intentionally, but owing to the phenotypic linkages they are inherited correlatively and therefore indirectly selected.

Concerning the sugar content of the stem, the high concentration in certain guinea sorghum varieties (up to 18.6% of brix) serves as interesting results to be exploited for varietal improvement. Nebié et al. (2009) found brix values up to 21.8 but specifically in sweet stem sorghums of Burkina Faso.

In general, the varietal diversity in this study was high, but it is difficult to compare it with that found in other countries like Ethiopia (Teshome et al., 1997) or in Kenya (Ngugi and Maswili, 2010) owing to the differences in the choice of the assessed traits and racial background.

The agromorphological diversity structuration of the 150 local sorghum varieties into three groups (Figure 4) was not based on village, botanical racial (except the sub-race guinea-margaritiferum varieties) or pericarp colour criteria. The structuration on the base of cycle duration (NHD) and grain productivity (GRW) shows clearly the importance of floral-biology on sorghum productivity, particularly under low rainfall conditions. Seven of the thirteen villages of this study have higher diversity representative in all the three groups structured by the HCA. Each one of these villages gives quite a good representation of the varietal diversity encountered in the North region of Burkina Faso and could constitute units of management for the preservation of varietal diversity on the scale of “terroirs”. Compared with other studies, Barro-Kondombo et al. (2010) reported that the cycle duration, plant height, 1000-grains weight and grain vitreousness were the principal structuring factor in local sorghum varieties from three regions of Burkina Faso. Koffi et al. (2011) reported in their study on the sorghums from the North of Côte d’Ivoire that the weight of 1000 grains, the cycle duration and the number of leaves were the first factors of the structuring of the diversity studied.

Conclusion

Assessment of the agromorphological diversity of the 150 local sorghum varieties from the North region of Burkina Faso showed that the varietal diversity cultivated by farmers was high and largely dominated by sorghums of the guinea botanical race, which indicates that racial configuration of sorghums remains unchanged in Burkina Faso. In terms of management, the three groups from the (HCA) are different entities based on phenology. However, for the North region, the Group 3 which contains late maturing varieties (76.6 days NDH) characterized by low productivity would be more exposed to the risks of abandonments, due to the rainfall constraints and probably parasitic attacks like midge (*Stenodiplosis sorghicola*). For this plant material, molecular analysis would be useful to confirm gene pools. Moreover, it is important to confirm the agronomic performance of the early maturing varieties, their tolerance to sorghum pests and other pathogens, their grain quality and even their biochemical composition with the aim of their use in the varietal improvement program.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Effectiveness of combining certain biotechnical methods with thymol treatment against *Varroa destructor* infestation

Mahir Murat Cengiz

Department of Plant and Animal Production, Erzurum Vocational School, Ataturk University, TR-25240, Erzurum, Turkey.

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In this study, the effectiveness of combining various biotechnical methods with thymol was investigated against the mite, *Varroa destructor* during late summer. Experimental colonies were randomly selected and six study groups were formed with nine colonies in each group. Experimental colonies were created as follows: colonies of renewed queen bees (RQ); colonies in which the queen is trapped on one comb, but worker bees can come and go to carry out their duties (CT); colonies in which ten grams of powdered thymol was added to 90 g of the bee cake, and 100 g of the bee cake with thymol was applied to the colonies (TY); colonies in which the requeen method plus the thymol method were used (RQ+TY); colonies in which the comb trapping method plus the thymol method were used (CT+TY); and untreated control colonies (CC). During the late summer period, the mite infestation level, sealed brood areas, bee population, and effectiveness of applications were determined in the groups. There was no significant difference in the infestation rate, sealed brood areas, and bee populations in the treatment groups before brood interruption. The efficacy of the requeen method, the comb trapping method, the thymol method, the requeen plus thymol method, and comb trapping plus other groups against *V. destructor* infestation were 40.23, 39.76, 80.45, 98.28 and 97.93%, respectively. These results showed that combining biotechnical methods with thymol is a safe, easy and effective alternative to late summer therapy against *V. destructor.*

Key words: Comb trapping, honey bee, requeening, thymol, *Varroa destructor.*

INTRODUCTION

The mite *Varroa destructor* has many deleterious impacts on honey bee colonies (Gunes et al., 2017). Therefore, mite infestation in honey bee colonies needs to be constantly controlled by acaricides. To date, synthetic compounds such as coumaphos, amitraz, fluvalanite and flumethrin have been used against mite invasion. However, these synthetic compounds leave residues in honey and honey wax, and mites gradually develop resistance to their active ingredient over time (Wallner, 1999; Medici et al., 2015). In recent years, soft acaricides

E-mail: mcengiz@atauni.edu.tr.

such as thymol which do not lead to mite resistance and do not show any toxic effect on honey bees (Gregorc et al., 2018), have started to be used worldwide (Imdorf et al., 1999; Cengiz, 2012; Giacomelli et al., 2016).

Thymol, found naturally in honey, has high acaricidal efficacy against mites when used both alone or in combination with other biotechnical control methods (Coffey, 2007; Giacomelli et al., 2016). Investigations show that the acaricidal effect of thymol is low in the presence of brood, but high in the absence of brood (Akyol and Yeninar, 2008; Rosenkranz et al., 2010). Thymol has been used by a number of researchers to reduce mite infestations (Calderone, 1999; Cengiz, 2012; Brasesco et al., 2016; Dar et al., 2017). According to Giacomelli et al. (2016), biotechnical methods are sustainable approaches for mite treatment. Biotechnological applications, such as brood removal and caging the queen are cause of artificially broodless period in honey bee colonies and were already successfully used to *V. destructor* infestation control in combination with products based on thymol (Calderone, 2005; Rosenkranz et al., 2010; Gregorc et al., 2017a). The techniques of requeening and comb trapping also allow one to create a broodless period in the colony to be created. A broodless period would interrupt mite population growth (Calis et al., 1999; Huang, 2001; Wagnitz and Ellis, 2010).

The first objective of this research was to determine effects of requeening and comb trapping on the efficacy of *V. destructor* control using thymol. The second aim was to determine the positive and negative effects of these procedures on the pre-winter performance of the colonies. The results of this research will enhance alternative application among beekeepers in their fight against *V. destructor* in honey bee colonies.

MATERIALS AND METHODS

This study was carried out on a total of 54 colonies including 45 experimental colonies and 9 controls in Narman, Erzurum (40°21'3.70" E longitude, 41°52'39.09" N latitude, and 1650 m above sea level). The experiment was conducted from August 11 to September 19, 2017. Before the experiment, the colonies were equalized for brood area (3879.45±70.94 cm^2), bee population (41883.18±453.15 pcs) and for the quantity of food (two frames full of honey and pollen). At the beginning of the research, all the colonies were queenright and had brood present.

The natural mite mortality was monitored three times, three weeks prior to the beginning of the experiment. Two weeks prior to the start of thymol treatment, the level of infestation of the experimental colonies was determined using sticky bottom boards. The colonies were randomly divided into six groups according to applied treatment; colonies with renewed queen bees (RQ); colonies where the queen is trapped on one comb, but worker bees can come and go to carry out their duties (CT); colonies where thymol in sugar bee candy was applied (TY); colonies in which the requeen plus thymol methods were used (RQ+TY); colonies in which the comb trapping plus thymol methods were used (CT+TY); and untreated control colonies (CC).

For the treatment with thymol, bee cake was prepared (18 kg Ice sugar + 3 kg honey + 400 g pollen + 2 L water). Ten grams of powdered thymol was added to 90 g of the bee cake. One hundred grams of the sugar bee candy with thymol was applied to TY, RQ plus TY and CT+TY groups. The thymol treatments were applied weekly for three weeks. The requeen, comb trapping, and control colonies received thymol free sugar bee candy.

In the requeening group, queen bees were caged at the beginning of the experiment. Old caged queens were removed from the hive after 20 days, and young mated queens were introduced to the colonies by using the cages. The queens, who were kept in the cage for one day, were released. In the comb trapping groups, the queens were imprisoned in a single frame and released after 21 days in the frame. Combs of caged queen bees were taken from the hive, and combs were destroyed. There were no drone brood in this period in the colonies, and, after 21 days, the sealed brood remained. These two treatments led to the emergence of sealed brood in the colonies prior to thymol treatment. The exposure of many mites to thymol therapy increased the efficacy of thymol. Approximately 300 bees were collected from each colony to determine the adult bee infestation level in the experimental colonies. Worker honey bees from the brood combs were brushed into glass jars from which the circular center portion of the lids had been removed and replaced with a 3.1 mm mesh screen (Gregorc et al., 2017b). Then, about two tablespoons (15 g) of powdered sugar was added to the bees, and the glass jar was turned for one min to ensure proper mixing of the powder sugar and honey bees. The honey bees were vigorously shaken over a white paper plate for about four min, and the dislodged *V. destructor* were then counted.

The capped brood area was measured by the PUCHTA method ($S = 3.14 \times A/2 \times a/2$) in cm^2 (Akyol et al., 2014; Cengiz and Erdoğan, 2017). Before and after treatments, the bee population was estimated as the number of combs covered with bees and multiplied by 2972 method by Gris Valle et al. (2004).

Oxalic acid treatment was used as a finisher treatment to test the efficacy of the treatments. 100 g of oxalic acid dihydrate were diluted in a 1:1 sugar solution (1000 g sucrose + 1000 mL water), and 4.2% oxalic acid solution was prepared. Oxalic acid solution was trickled using a large-scale syringe so that a 5 mL solution would be placed on each comb covered with bees (Gregorc et al., 2016).

Climatic temperatures were recorded during the trial period for the evaporation of thymol in parallel with the increase in temperature (Imdorf et al., 1999). Thymol is most efficacious at a temperature range between 15 and 35°C. Air humidity was not recorded, as thymol evaporation is not influenced by this parameter (Giacomelli et al., 2016). Sucrose syrup was prepared at ratio of 2:1 autumn feeding and each trial colony was given 0.5 L syrup for the 15-day autumn feeding period.

Statistical analysis

The SPSS 20.0 for Windows package program was used for statistical evaluation, and multiple comparison tests were performed for significant components. Prior to the analysis of variance, regarding the arcsine$\sqrt{y/100}$ transformation to the percent efficiency of the organic compounds used in the investigation; a logarithmic transformation (log10) was applied to the amount of the sealed brood area and a bee population that did not fit the normal distribution.

RESULTS AND DISCUSSION

The external temperatures in the tested apiaries mostly remained within the optimum temperature range described for thymol during the 20-days of treatment

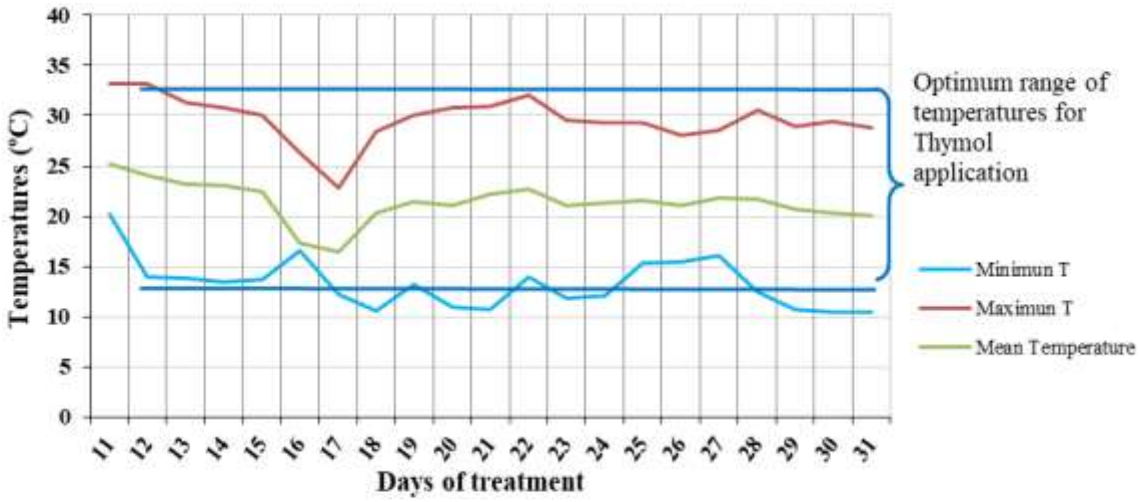

Figure 1. Temperatures recorded in Erzurum city during the trial (°C).

Table 1. Number of mites on bees and infestation rate on bees.

Treatment	n	Number of mites on bees at various stages of the experiment ($\bar{x} \pm S_{\bar{x}}$)		Infestation Rate on Bees (%) ($\bar{x} \pm S_{\bar{x}}$)		
		Before brood interruption	After three weeks of brood interruption	Before brood interruption	After three weeks of brood interruption	After thymol treatments
RQ	9	10.22±1.53	50.88±6.38^{a}	3.40±0.51	16.96±2.12^{a}	14.85±0.96^{a}
CT	9	9.88±1.64	47.77±5.72^{a}	3.29±0.54	15.92±1.91^{a}	14.55±0.78^{a}
TY	9	11.33±1.96	13.55±2.17^{b}	3.77±0.65	5.81±0.80^{b}	4.22±1.16^{b}
RQ + TY	9	10.11±1.98	49.11±7.42^{a}	3.37±0.66	16.37±2.47^{a}	1.44±0.66^{c}
CT+TY	9	8.66±2.23	45.22±6.49^{a}	2.88±0.74	15.07±2.16^{a}	1.51±0.62^{c}
CC	9	8.22±1.78	12.67±2.22^{b}	2.74±0.59	5.40±0.82^{b}	15.88±2.36^{a}
Total	54	9.74±0.74	36.53±3.13	3.61±0.26	12.59±0.98	8.74±0.91

^{a,b,c}Means with no common superscript in a column within a parameter differ (P<0.05).

(Figure 1). In fact, the mean temperature was 22.4°C and the maximum was never over 35°C. However, the mean minimum temperature during the trial was 13.8°C. This mean temperature was slightly below ideal temperature (15°C).

There was no significant difference in the infestation rate and number of mites on bees among the treatment groups before brood interruption ($F_{5.48}$= 0.36; *P < 0.01*). The treatment groups ranged from a high of 10.22±1.53 mites on bees to a low of 8.22±1.78 mites on bees. However, there was an increase in the number of *Varroa* on adult bees in the break in the brood cycle groups after three weeks ($F_{5.48}$ = 11.06; P < 0.01, Table 1). The difference in the increase in number of mites observed between groups was also found to be statistically significant ($F_{5.48}$ = 11.06; P < 0.01). Thymol treatment samples showed that the mite infestation rate decreased significantly in the requeen plus thymol and comb trapping plus thymol treatment groups. The infestation rate decreased from 16.37±2.47% to 1.44±0.66% and 15.07±2.16% to 1.51±0.62% for requeen plus thymol and comb trapping plus thymol group, respectively.

The total number of mites fall during treatment was different from the other groups in the requeen plus thymol and comb trapping plus thymol groups. The differences in the weekly mite fall observed between groups were also found to be statistically significant ($F_{5.48}$ = 163,98; P<0.01). The lowest mite fall after the application of oxalic acid was determined in the requeen plus thymol (10.33±0.52) and comb trapping plus thymol (12.33±0.89) groups (Table 2).

There was no significant difference between the brood area ($F_{5.48}$ = 0.31; P < 0.01) and bee populations ($F_{5.48}$ = 0.66; P < 0.01) of the pre-treatment colonies, while the difference in brood areas of the requeen and the requeen plus thymol groups after treatment was statistically

Table 2. Number of fallen mites during treatment with thymol and oxalic acid.

Treatment	n	Number of mite fall during treatments			Total mite fall ($\bar{X} \pm S_{\bar{x}}$)	Total mite fall with oxalic acid ($\bar{X} \pm S_{\bar{x}}$)
		1st week ($\bar{X} \pm S_{\bar{x}}$)	2nd week ($\bar{X} \pm S_{\bar{x}}$)	3rd week ($\bar{X} \pm S_{\bar{x}}$)		
RQ	9	72.66±4.78[b]	50.77±2.39[c]	74.77±4.43[bc]	198.22±9.36[c]	294.22±7.98[b]
CT	9	70.66±3.80[b]	48.77±2.13[c]	72.66±3.41[bc]	192.11±7.62[c]	297.33±7.92[b]
TY	9	284.66±19.12[a]	131.44±8.42[b]	82.77±7.34[b]	498.88±21.74[b]	121.22±2.69[c]
RQ + TY	9	321.66±19.89[a]	162.44±7.31[a]	106.77±7.60[a]	590.88±21.91[a]	10.33±0.52[d]
CT +TY	9	316.67±18.22[a]	161.88±8.39[a]	104.77±6.91[a]	583.33±19.91[a]	12.33±0.89[d]
CC	9	61.66±4.11[b]	39.77±2.03[c]	63.66±4.11[c]	165.11±8.39[c]	315.22±7.98[a]
Total	54	188.00±17.37	99.18±7.74	84.24±3.19	371.42±26.69	175.11±18.33

[a,b,c,d]Means with no common superscript in a column within a parameter differ (P<0.05).

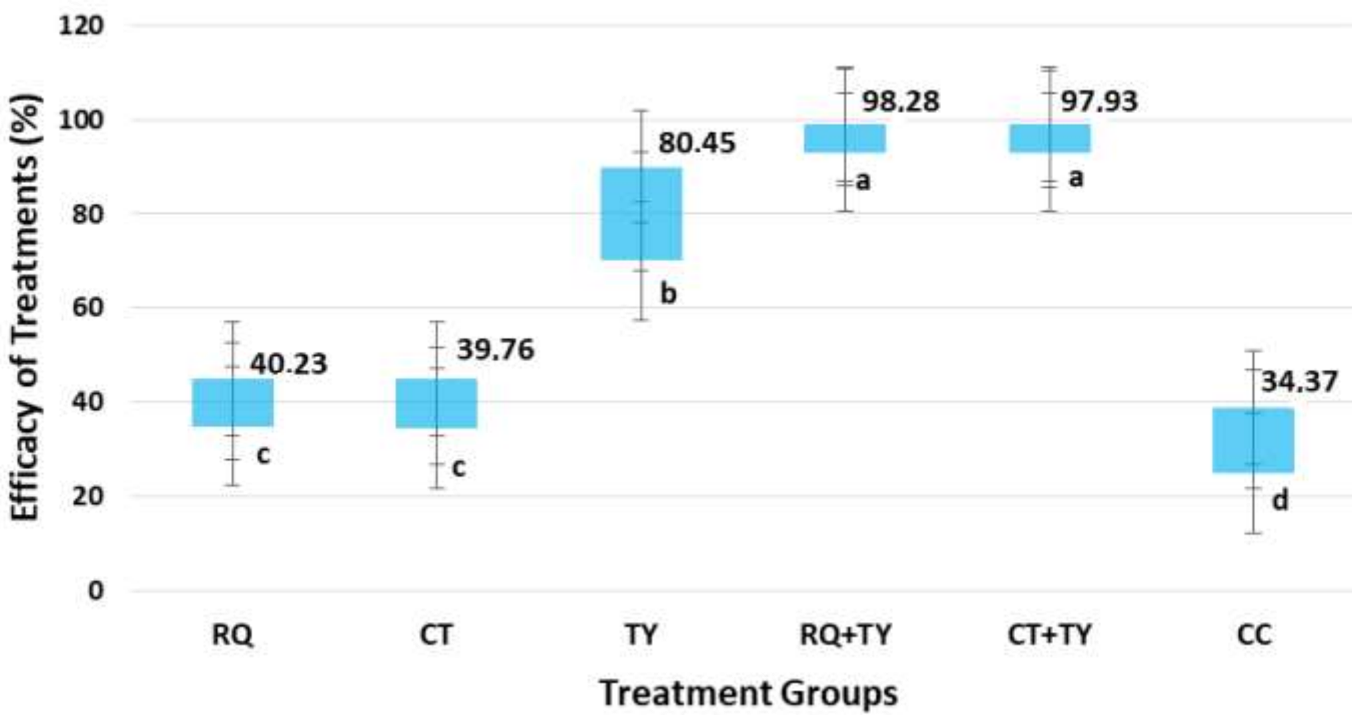

Figure 2. Efficacy of treatment groups on control of *Varroa destructor*.

significant ($F_{5.48}$ = 27.85; P < 0.01). On the other hand, there was a decrease in the bee population in all groups except in the thymol and control groups (Table 3). The differences in the bee population between the observed groups were also found statistically significant ($F_{5.48}$ = 21.24; P < 0.01). The differences in treatment efficacy between the observed groups were also found to be statistically significant ($F_{5.48}$ = 874.25; P < 0.01).

The reduction rates of mite infestation for the requeen and comb trapping groups alone were determined as 40.23 and 39.76%, respectively. The treatment efficacy of requeen plus thymol (98.28%) and comb trapping plus thymol (97.93%) was different from other methods (Figure 2).

The application of the requeening and comb trapping methods in late summer reduced the mite destruction level in honey bee colonies. These two methods were not as effective as co-administration with thymol, although they caused a significant reduction in *V. destructor* infestation. The efficacy rate was 80.45% for the colonies treated with thymol. The average efficacy of thymol in the experimental colonies were found to agree with previously reported results by Emsen et al. (2007), (informed as 83.15%), but were lower than the result of another study Montano and Guzman-Novoa (2007), (informed as 92.1%). The average efficacy of thymol in the present study was higher than in previously reported finding by Cornelissen and Gerritsen (2006), (informed as

Table 3. Effects of applications on sealed brood areas and bee populations.

Treatment	n	Sealed brod area (cm^2)		Number of bee population	
		Before Treatment ($\bar{X} \pm S_{\bar{X}}$)	After Treatment ($\bar{X} \pm S_{\bar{X}}$)	Before Treatment ($\bar{X} \pm S_{\bar{X}}$)	After Treatment ($\bar{X} \pm S_{\bar{X}}$)
RQ	9	3931.10±176.71	1592.88±59.34[a]	42598.66±1213.31	25757.33±495.33[b]
CT	9	3798.43±167.95	1103.37±26.56[b]	40947.55±1289.55	25096.88±522.12[b]
TY	9	3960.98±171.75	1081.55±34.62[b]	42268.44±1082.70	30380.44±660.44[a]
RQ + TY	9	3984.65±202.84	1606.22±73.29[a]	42928.88±522.12	25922.44±436.84[b]
CT +TY	9	3873.43±185.28	1092.87±29.32[b]	41938.22±1044.25	25427.11±459.64[b]
CC	9	3728.10±171.46	1064.83±59.81[b]	40617.33±1401.01	29389.77±330.22[a]
Total	54	3879.45±70.94	1256.95±38.64	41883.18±453.15	26995.66±344.14

[a,b,c,d]Means with no common superscript in a column within a parameter differ ($P<0.05$).

70.7%). This is thought to be related to the colonies' sealed brood areas. This research shows that artificial brood interruption with requeening and comb trapping applications is effective in increasing the efficacy of thymol treatment. However, the number of varroa on the bees increased in the groups in which the brood was artificially interrupted. Actually, the report that a large majority of the *Varroa* mites were in the sealed brood was consistent with the results of this study (Mondet et al., 2018).

The average efficacy of the requeen plus thymol and the comb trapping plus thymol groups was calculated as 98.28 and 97.93%, respectively. The efficacy values of these groups in the study are consistent with previously reported result by Giacomelli et al. (2016) (informed as 96.8%). At the end of the study, the decrease in the population of bees was considerably higher in the break in the brood cycle groups than in the other groups. This is due to the artificial brood interruption of brood production. In the requeen and requeen plus thymol groups, the sealed brood area after treatment was significantly higher than in the other groups. In other words, the sealed brood areas in the requeening have increased. Many researchers have reported that young queen bees produce more brood (Woyke, 1984; Akyol et al., 2007; Koç and Karacaoğlu, 2011; Öztürk, 2013; Büchler et al., 2013).

Conclusions

Colony management techniques used in this study have many advantages in the fight against *V. destructor*. The results show that an artificial brood interruption in the brood cycle can be used successfully to enhance the efficacy of different treatments. Particularly with the requeening combining thymol application, beekeepers will not only be successful in the fight against the *V. destructor* but also have young queen bee for the next season. When an adequate autumn feed is made in the requeen colonies, the production of brood in the colonies can be increased, and the colonies can be successfully overwintered. As a result, the application of the requeen plus thymol and comb trapping plus thymol methods efficacy against *V. destructor* was found similar. However, it can be said that the requeen plus thymol method is more advantageous than the comb trapping plus thymol method in terms of increasing brood production.

CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

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