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Effects of soil texture and burial depth on the biological parameters of overwintering pupae of *Bactrocera oleae* (Diptera:Tephritidae)

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This study investigates the effect of soil texture and pupal burial depth on some selected bio ecological parameters of *Bactrocera oleae* (Diptera: Tephritidae), as one of the major pests of the olive tree. Thus, we focused on the emergence rate of adult flies, the duration of pupation and the parasitism rate by *Psyttalia concolor* (Hymenoptera: Braconidae). The three types of soils determined (clay, sandy-loam and Sandy-Clay-Loam) were tested at 6 depths ranging from 2 to 16 cm. The results have showed that the duration of pupation, the emergence rate and the postnatal mortality of this pest are influenced by the soil texture and the burial depth of its pupae. Regarding sandy-loam soil, we obtained an average emergence rate of 46.31%, whereas in the clay soil and Sandy-Clay-Loam soils this rate was about 38%. The post-emergence mortality rate was higher in the clay soil, at 16 cm depth. The duration of pupation is proportional with the burial depth of the pupae.

Keywords: ***Bactrocera oleae*, pupation, emergence, burial depth, soil texture.**

INTRODUCTION

The olive tree (*Olea europaea* L.) is historically known to be a very old tree and a classical feature of the Mediterranean landscape (Cherubini et al., 2013). Despite its hardiness, the olive tree is subject to attacks by several pests causing a significant reduction in production. Among these pests, the olive fruit fly, *Bactrocera oleae* (Rossi) (Diptera: Tephritidae) is considered as the main pest of olives in the world (Daane and Johnson, 2010).

Various aspects of related to its biology, ecology, the management of its populations and its impact on olive production have been described by many authors (Ait Mansour et al., 2015). Chemical control, using organo-phosphorus and pyrethroid insecticides, has been the predominant method, although its dangerous effects on humans and the environment are well known (Murphy, 1986). Nevertheless, the discovery of insecticide resistance among some of the populations (frequently treated with these insecticides), has prompted scientists to search for new biological and cultural methods to effectively control this pest.

Today's consumers demand that farmers care about human health and the environment, and these requirements result in an increased request for environmentally friendly organic products, i.e. without the use of inputs.

Indeed, several techniques have been advocated as means of biological control to limit *B. oleae* populations. We can mention the clays (kaolinite) successfully used in some olive-growing countries as a mean of physical control (Pascual et al., 2010) and auxiliary plants such as the viscous inula, used in biological control by conservation. In fact, this plant hosts a host named, *Myopites stylata* (Diptera, Tephritidae), which is the parasite of the olive fruit fly, *Eupelmus eurozonus* (Hymenoptera, Eupelmidae). Indeed, the viscous inula is attacked by *M. stylata* which causes galls under the inflorescences. This fly may be parasitized by *E. eurozonus* which also attacks *B. oleae* (Warlop, 2006).

Cultivation practices that do not require a lot of resources can be very effective in reducing *B. oleae* populations. Soil tillage can reduce populations of Tephritids that pupate in the soil. It is in this context that our work focuses to study the effect of soil texture and burial depth of pupal on some bio ecological parameters of *B. oleae* under laboratory conditions.

MATERIALS AND METHODS

Soil sampling and physicochemical analyzes

At the three experimental sites we performed random soil sampling. The work involves taking 5 squares of 20 cm side and 10 cm deep. The collected soil is transported in plastic bags to the laboratory. The 5 samples of each soil were mixed, dried and milled, and sieved using a sieve of 2 mm diameter to obtain fine soil. The sieved soil received a granulometric analysis whose principle consists in the destruction of the organic matter by an attack with oxygenated water. The particles are then dispersed with sodium hexametaphosphate. We adopted an international method that uses Robinson's pipette by taking samples at different depths and at specific times (Baize, 2000). The Carbon and organic matter were estimated using Anne's method (1945), the principle of the latter consists of the titration of the unreacted acid with potassium dichromate ($K_2 Cr_2 O_7$) in a sulfuric medium. Residual humidity was obtained at 105°C.

Collection of pupae

In order to use pupae of the same age, we took the larvae during the olive harvest period. Noting that in the study area this is done using plastic nets. At the time of harvest, we collected a large number of *B. oleae* larvae that were put in Petri dishes, and after 24 hours, we retrieved the pupae of the same age with which our tests were conducted.

Tests of textures and depths in the laboratory

We tested six (06) depths for the three soil types namely: Dp1 (1 cm), Dp2 (4 cm), Dp3 (7 cm), Dp4 (10 cm), Dp5 (13 cm) and Dp6 (16 cm). For each depth we have provided a control. Four repetitions were performed for each depth, including the control.

Twenty pupae are placed at the bottom of the plastic bottles of 10 cm in diameter and were buried by the sterilized soil whose height varies from 1 cm to 16 cm. The bottles are then covered with muslin. The control sample consists of placing 20 pupae at the bottom of a device without covering them with a layer of soil. From the first emergence, the number of adults is counted daily in order to estimate the emergence rate and the duration of pupation. At the end of the emergence of adult flies, we sieved the soil to highlight the post-natal mortality rate and the rate of true parasitism by *Psyttalia concolor* (Hymenoptera: Braconidae). This Chalcidians is native to the eastern part of the Mediterranean Basin. It is also found on other tephritids attacking the argan tree, wolfberry, caper or jujube, in the Maghreb (Fischer, 1971).

Laboratory uncontrolled conditions (mean temperature and relative humidity) under which our experiments have been made are mentioned in table 1.

Statistical analysis of the results

The obtained results have been assessed using the analysis of variance (Anova) test with three criteria of classification and the test of Newman and Keuls. These methods compare the averages of several populations, assumed to have of normal distribution and of the same variance, resulting from random samples that are simple and independent (Dagnelie, 1973, 1975).

RESULTS

Granulometric analysis of the three soils tested

The results of the particle size analysis show that the three soils have similar levels of organic matter. Soil 1 consists of a large amount of clay. Soils 2 and 3 are composed of a large portion of sand. Nevertheless, soil 2 is composed of 26.11% fine sand and soil 3 contains only 4.61% fine sand (Table 2).

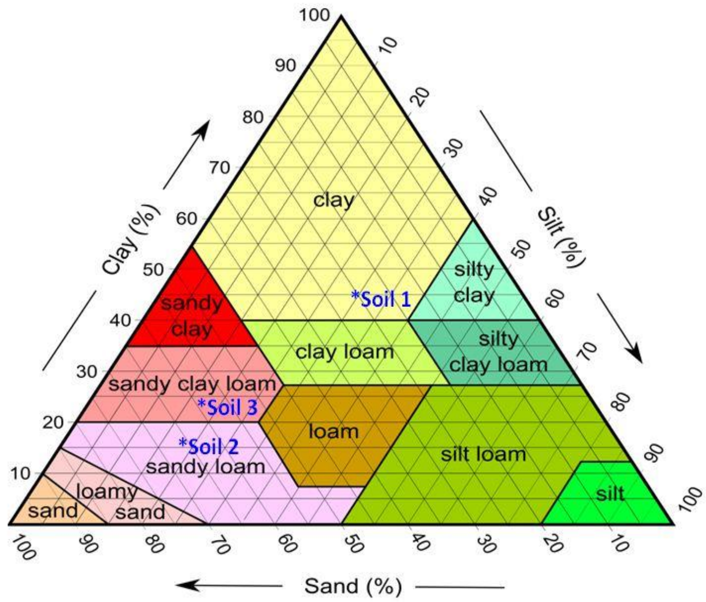

Figure 1: Texture diagram used by the United States Department of Agriculture showing the three types of soil.

Table 1: mean temperatures (° C) and relative humidity (%) recorded in the laboratory during the experimental period

		December	January	February	March	April	May
Mean temperature ± Standard deviation	2013/2014	14.8±3,2	17.35±2	15,23±1,99	19,36±3	21,95±1,9	23,9±0,99
	2014/2015	12,9±1,89	15,44±1,78	14,8±2	17,7±2,8	20,5±0,9	22,89±1
	2015/2016	14,5±2,6	16,75±1	16,23±1,66	18,35±1,99	22,6±1	23,5±1,33
Mean relative humidity ± Standard deviation	2013/2014	74,4±0,8	61,77±1,6	61,52±3,9	55,82±5,6	58,25±7,3	59±4,2
	2014/2015	73,4±5,8	60,8±6,7	60,8±4	56,6±8,9	57,7±10,01	58,6±7,5
	2015/2016	75±4,8	59±5,9	58±7,19	56±7,66	57±7,99	57±7,89

Table 2: Results of the analyzed soils

Parameters	Soil 1	Soil 2	Soil 3
Residual humidity (%)	3.93	2.79	2.8
Organic matter rate (%)	3.7	2.72	3.05
Total limestone rate (%)	5.23	5.85	5.23
Silt (%)	31,77	23,46	25,49
Clay (%)	44,81	16,58	20,96
Sand (%)	23.42	59.95	53.62
Soil texture	Clayey	Sandy loam	Sandy clay loam

The texture diagram ranks soil 1 as clay, soil 2 as sandy-loam and soil three as Sandy-Clay-Loam (Figure 1).

II. 2. Effect of soil texture and burial depth on the emergence rate of *B. oleae*

For three types of soil, we have noticed that the emergence rate of the olive fruit fly decreases significantly when the depth of pupal burial increases (F=163.67; ddl=6; P = 0). On the other hand, the effect of factor year is insignificant (F=0.702; ddl=2; P = 0.5). The emergence rate varies between 40.01 and 41.72% (Table 2). Soil texture has a very high significant effect on the emergence rate (F=18.97; ddl=2; P = 0).

The Newman and Keuls test, at the 5% threshold, classifies the three studied textures into two homogeneous groups. Group A includes sandy-loam soil with a rate of 46.31%. Group B corresponds to clay and sandy clay loam soils with rates of approximately 38% (Table 3).

The depths tested were classified by the Newman and Keuls test in 5 homogeneous groups. Group A includes depth 1 cm with the highest emergence rate (64.02%). The 16 cm depth generated the lowest emergence rate, with 5.02% (Table 3).

The results highlighted that soil texture and larval burial depth have a very high significant effect on the emergence rate of *B. oleae*.

Under sandy loam soil, we recorded an average emergence rate of 46% and a post-emergence mortality rate of 7%. On the other hand, at the clay soil level, an emergence rate of 38% and a post-emergence mortality rate of 12.78% were obtained; this might be due to the presence of clay. Indeed, Prone (2003) and Baize and Jabiol (1995) point out that clayey soils are considered agronomically heavy. The predominance of clay makes this soil more coherent and more asphyxiating, for the lack of O_2, adults individuals die before reaching the surface.

The sandy-clay-loam soil also generated a low emergence rate of 38.33% and a post-emergence mortality rate of 9.44%. This is probably due to the asphyxiating particle size of the silts and clays that is accentuated by sand particles, making this texture heavier and harder for adult individuals. Baize and Jabiol (1995) wrote that the presence of sand in the sandy-clay-loam texture is very well detected, but it is not dominant. According to Prone (2003), the presence of fine sands tends to fill hols and causes compaction. Sand associated with silt increases the density of this texture. Moreover, Dajoz (1975) reported that other factors can also intervene such as the porosity and consequently the quantity of oxygen available, they can be involved as factors limiting the distribution of the larvae.

The results are similar to those obtained by Zerkhefaoui (1998), which recorded an emergence rate ranging from 62 to 69%. However, Hamiche (2005) had an emergence rates ranging from 73.3 to 90.9% in October. According to the same author, this rate does not exceed 37.5% in December, in the olive groves of the region of Tizi-Ouzou (Algeria).

For the burial depth of pupae, the rate of emergence decreases as the burial depth increases. The highest rate (64.02%) was obtained with depth 1 cm. The depth of 4 cm and the control are classified in the same homogeneous group with respective rates of 59.16% and 55.27%. The 16 cm depth allowed an emergence of only 5.02% for the pupae.

II. 3. Effect of texture and depth of burial on *B. oleae* post-emergence mortality

The post-emergence mortality rate varies between 8 and 10% in a non-significant manner (F= 1.276; ddl= 2; P = 0.28), for the three tests carried out. In contrast, soil texture and larval burial depth act very highly (F=7.488; ddl=2, P = 0.0009 and F=16.922; ddl=5 P=0, respectively).

The highest mortality rate (12.78%) was recorded in the clay soil. The latter was classified by the Newman and Keuils test in group A. sandy loam and sandy clay loam soils were classified in the same homogeneous group (B) with average rates of 9.44 and 7.08% respectively (Table 4). Regarding the larval burial depth, the highest mortality rate (17.64%) was observed at depth 16 cm. The latter was classified by the test of Newman and Keuils in the first homogeneous group (A). The depths 1 and 4 cm generated only 2.78% and were classified in the last homogeneous group C (Table 4).

The post-emergence mortality rate is proportional to the depth of pupal burial. A mortality of 17.64% was recorded under the depth 16 cm and only 2.78% were obtained for the depths 1 and 4 cm. According to Liaropoulos (1978), the type and structure of the soil affect the mortality of *B. oleae* pupae. Thus in Crete, in a region with grainy and well-drained soil, the percentage of survival increases progressively from 19% in November to 47% in March. On the other hand, in heavy soil, it is 30% in September, and tends to stabilize at a lower level (0-20%) until March-April. In addition, Orphandis (1958) reports that pupal mortality varies greatly by region, date of burial, climatic and soil conditions. The lowest percentages of mortality were generally observed for larvae that burrow early in the fall and emerge before winter, compared to those buried in early winter, whose adults do not emerge before the next spring.

Table 3: Newman & Keuls test results concerning the effect of soil texture and burial depth on the emergence rate of *B. oleae* adults.

Factor	Type	Mean ± SD
Test	Test 1 (2013) Test 2 (2014) Test 3 (2015)	41.72 ± 8.34 41.25 ± 8.51 40.01 ± 8.44
Soil texture	Sandy loam Clayey Sandy clay loam	46.31 ± 7.50 (A) 38.34 ± 10.26 (B) 38.33 ± 7.19 (B)
Depth (cm)	1 Control 4 7 10 13 16	64.02 ± 10.99 (A) 59.16 ± 8.82 (B) 55.27 ± 10.24 (B) 41.38 ± 9.25 (C) 31.94 ± 8.0 (D) 30.13 ± 5.92 (D) 5.02 ± 3.58 (E)

Table 4: NEWMAN & KEULS test results for the effect of soil texture and pupal burial depth on post-emergence mortality of *B. oleae* adults

Factor	Type	Mean ± SD
Test	Test 1 (2013) Test 2 (2014) Test 3 (2015)	10,97 ± 7.73 9,72 ± 8.72 8,61 ± 6.61
Soil texture	Clayey Sandy clay loam Sandy loam	12,78 ± 8.98 (A) 9,44 ± 7.44 (B) 7,08 ± 6.59 (B)
Depths (cm)	16 13 10 7 1 4	17,64 ± 1.65 (A) 13,47 ±1.8 (AB) 13,05 ± 1.74 (AB) 8, 89 ±1.6 (B) 2,78 ± 1.17 (C) 2,78 ± 1.26 (C)

Similar results have been obtained by other authors working on another species of Tephritidae as *Ceratitis capitata*. For instance, Cavalloro and Delrio (1978) report that the pupation of this pest does not depend on the chemical composition of soils, but on their texture. Ali Ahmed Sadoudi (2007) and Ali Ahmed et al., (2007) also report that the rate of emergence decreases very significantly as larval burial depth increases. Metna (2009) wrote that soil texture has a significant effect on the adult emergence rate of *C. capitata* and that the silty texture allows for the highest emergence rate. However, this author added that neither the texture of the soil nor the depth of burial of the pupae act on the pupation duration of *C. capitata* adults.

II.4. Effect of soil texture and pupal burial depth on pupation time of *B. oleae*

The pupation duration of the olive fruit fly varies insignificantly (F= 0.667; ddl = 2; P = 0.52) for the three tests performed. For the depth factor, the pupation duration tends to increase very highly when the depth of pupal burial increases for all three soil types (F=75.781; ddl=6; P = 0). Similarly, the analysis of the variance has showed a very high significant difference (F=11.409; ddl =2; P = 0.0003) for the mean *B. oleae* pupation durations for the three studied textures.

The pupation time is proportional to the burial depth. The 16 cm depth generated the longest duration with 75.6 days. It is classified with depth 13 cm in the homogeneous group A. The control and depth 1 cm are classified in the same homogeneous group D, with respective durations of 43.55 and 35 days (Table 5).

Table 5: Newman and Keuls test results showing the effect of soil texture and burial depth on the pupation time for *B. oleae.*

Factor	Type	Mean ± SD
Test	Test 1(2013)	53.91± 4.12
	Test 2 (2014)	54.11 ± 2.28
	Test 3 (2015)	56.25 ± 3.11
Depths (cm)	16	75.60 ± 4.65 (A)
	13	73.51 ± 2.54 (A)
	10	60.48 ± 4.21(B)
	7	51.66 ± 4.61(C)
	4	48.22 ± 0.56 (CD)
	Control	43.55 ± 1.16 (D)
	1	35.99 ± 3.30 (D)
Texture	Calyey	59.84 ± 2.90 (A)
	Sandy loam	54.34 ± 4.13 (B)
	Sandy clay loam	52.54 ± 2.51 (B)

The Newman and Keuls test, at the threshold of 5%, classifies the three textures into two homogeneous groups. Group A includes the clay soil that gave rise to the longest pupation time (59.84 days). The sandy-loam soils and the sandy clay loam soils are classified in group B, with respective durations of 54.34 and 52.54 days (Table 5).

The results on the effect of soil texture and depth of pupal burial on pupation duration show that their action is highly significant under laboratory conditions. The sandy clay loam soil produced the shortest pupation duration of 52.54 days and was classified with sandy-loam soil in the same homogeneous group. For the clay soil, the pupation time was extended by 7 days. The shortest duration (35.55 days) was obtained under the depth of 1 cm. For the control (0 cm), the pupation duration was 43.99 days. By comparison, we can conclude that the duration of pupation was extended by 8 days. The longest pupation duration of 75.60 days was recorded under the depth of 16 cm.

Zerkhefaoui (1998) had obtained shorter pupation times ranging from 17 to 54 days. After 54 days, there was no more emergence of *B. oleae* adults from pupae collected in November. According to Michelakis (1980), *B. oleae* pupae buried in the soil during the month of October develop during 30 days. During the months of November and December, development times are 80 and 90 days respectively. In this context, it should be remembered that the pupae used for our experimentation are all formed during the second half of December. Dimou et al., (2003) report that the majority of *B. oleae* larvae pupate in the first three centimeters of soil and that pupation depths differ according to soil moisture, soil type and the interaction temperature-soil type-soil moisture. This explains why at a depth of 1 cm, the pupation time is shorter than at 0 cm. In fact, hygroscopic humidity and temperature may affect the conditions of nymphal development in the soil.

The results confirm the findings of the authors who studied the hypogeal phase of the olive fruit fly. Indeed, Bachouche (2009) recorded the shortest duration under a sandy-loam soil and the longest duration at a depth of 15 cm. Likewise, Hamiche (2005) obtained, under natural conditions, in a soil of loam texture, 70% of pupae between 0 and 5 cm, 28% between the depth 6 and 10 cm and 2% between 11 and 15 cm. For the loam-clay texture, the same author obtained 87.2% of pupae at a depth between 0 and 5 cm, 12.8% between 6 and 10 cm and no pupa between 11 and 15 cm. Liaropoulos (1978) showed that most pupae are observed just below the surface, between 0 and 4 cm, which corresponds to the part most easily penetrated by the larvae before the formation of the pupae. Additionally, AL-Zaghal and Mustapha (1987) wrote that the soil fraction between 2.5 and 7.5 cm is mostly penetrated by migrating larvae.

To explain the variation over time in the emergence of adults from larvae buried simultaneously in the same place, the existence of a diapause within just part of the population was considered, but it does not seem necessary. The depth of the location of the pupae in the soil, the physical structure and the humidity of the soil sufficiently influence the temperature at which the pupa is exposed and the fluctuations of the temperatures around the lower thermal threshold, even if they are not very important, can already play a big role. Thus, a 40-day difference in pupal duration for pupae of the same group, exposed to the sun or in the shade of a tree, could be observed (Laudeho et al., 1975).

II.5. Adult sex ratio of *B. oleae*

The sex ratio is in favor to females in the different trials. It generally varies from 0.51 to 0.71 (Table 6).

Table 6: Sex-ratio of *B. oleae* adults emerged for all tested depths

Year	Sex-ratio (soil 1)	Sex-ratio (soil 2)	Sex-ratio (soil 3)
2013	0.55 ± 0.01	0.51 ± 0.2	0.56 ± 0.12
2014	0.71 ± 0.26	0.63 ± 0.007	0.59 ± 0.09
2015	0.66 ± 0.03	0.54 ± 0.10	0.65 ± 0.4

For the sex ratio, the results showed that this parameter is in favor of females. Indeed, it varies between 0.55 and 0.71. The results confirm similar results of Bouktir (2003) and Hamiche (2005) who obtained a sex ratio of 0.6 for females caught in traps. On the other hand, Zerkhefaoui (1998) wrote that the sex ratio of the captured population is balanced, and it is 0.49. In fact, several parameters including climatic conditions and larval food can influence the sex ratio of *B. oleae.*

II.6. Parasitism rate of the pupae put under observation

The parasitism rates of larvae of the olive fruit fly are relatively low, ranging from 12 to 20% (Table 7). The analysis of the variance showed that this rate varies very highly according to the test per years (F=8.412; ddl=2; P = 0.0004).

In addition, we reported that during soil sieving, no post-emergence mortality of the *P. concolor* parasite was observed. The rate of parasite emergence is not influenced by landfill depth and soil texture.

The Newman and Keuls test, at the threshold of 5%, classifies the three years in two homogeneous groups. Group A includes parasitism rates recorded in 2014 and 2013, with respective rates of 20.47 and 16.9%. Parasitism rate of 12.08% was observed in larvae harvested during 2015 (homogeneous group B) (Table 7).

Parasitism results reveal that rates vary very highly significantly between 12.08 and 20.47% (depending on the year). The same results show that these results do not depend on the depth of pupal burial or the texture of the soil; which supports the results of the effect of soil texture and pupal burial depth on the emergence rate of *B. oleae.*

Table 7: Newman and Keuls test results for the effect of soil texture and depth of burial on the parasitism rate of *B. oleae* larvae.

Factor	Type	Mean ± SD
Test	Test 1 (2014)	20.47 ± 13.78 (A)
	Test 2 (2013)	16.90 ± 10.02 (A)
	Test 3 (2015)	12.08 ± 10.22 (B)
Depths (cm)	Control	16.11 ± 11. 24
	1	16.38 ± 11.41
	4	18.61 ± 13.93
	7	19.02 ± 10.78
	10	15.13 ± 13. 07
	13	17.22 ± 9.96
	16	12.91 ± 10.91
Soil Texture	Sandy loam	15 ± 11.71
	Clayey	16.96 ± 10.90
	Sandy clay laom	17.5 ± 12.13

The obtained results confirm the findings of Zerkhefaoui (1998) in Beni-Douala (Tizi-Ouzou, Algeria), he recorded a rate of parasitism of 16.7%. Moreover, Guaour (1996) obtained rates ranging from 9 to 46%. The latter justified the limitation of the populations of the parasite could be linked to the climatic conditions, especially the low hygrometry which would be unfavorable for the development of this auxiliary. In Aguistri (Greece), the parasitism rate of *B. oleae* pupae by *P. concolor* fluctuates between 12.5 and 75% (Canard et al., 1979).

CONCLUSION

This study has highlighted the importance of *B. oleae* activity. In fact, a significant number (1680 of same-age larvae) were sampled to perform a single test. The results show that emergence and nymphal development are dependent on soil texture and depth of pupal burial. We have found that the sandy-loam soil is favorable for the development of *B. oleae* pupae. The nature of this soil allowed obtaining a relatively high emergence rate (46.31%), than those obtained in clay soil (38.34%) and sandy-clay loam soil (38.33%).

The post-emergence mortality rate is higher in the clay soil, at a depth of 16 cm. As a result, we can suggest that tillage (deep tillage) could contribute in part to the elimination of overwintering pupae. The average pupation time varies from 35 to 75 days. Emergences generally range from 33 to 79 days. The longest times have been obtained in a clay textured soil at a depth of 16 cm. Parasitism results reveal the insignificant effect of soil texture and pupal burial depth on this parameter. But, it must be emphasized that parasitism can eliminate up to 20% of overwintering pupae.

It would be interesting to complete this work by making field trials for the aim to highlight the synergistic effect of soil texture and structure towards the bio ecological parameters of *B. oleae*.

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CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest".

AUTHOR CONTRIBUTIONS

B.N. designed and performleed the experiments and also wrote the manuscript. K.A. Contributed to the elaboration of the experimental plan and corrected the manuscript. L.S. performed translated into English. All authors read and approved the final version.

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