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Bio efficacy of *Ocimum sanctum* L. (Lamiaceae) leaf extracts against Pulse beetle (*Callosobruchus chinensis* L.) (Coleoptera: Bruchidae) in stored green gram (*Vigna radiate* L.)

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Abstract
A laboratory experiment was conducted in the laboratory of Plant Physiology, Department of Entomology, Assam Agricultural University, Jorhat during the year 2014, October to April, 2015 to evaluate the efficacy of *Ocimum sanctum* L. against *Callosobruchus chinensis* L. (Coleoptera: Bruchidae). Plants were extracted with petroleum ether, methanol, ethanol and water by using Soxhlet apparatus and tested against adult of *C. chinensis*. The leaf extract of *O. sanctum* were evaluated for their adult mortality, oviposition deterrence and adult emergence of *C. chinensis*. The results revealed that, among the solvent extracts petroleum ether was found to be significantly superior over rest of the solvent extracts, registered the highest percent of mortality (84%) at 5% conc. after 96 hour of treatment followed by methanol (82%), ethanol (80%) and water extract (76%) respectively. Highest oviposition deterrence was found in petroleum ether extract (60.75%), followed by methanol (59.38%), ethanol (56.22%) and water extract (50.11%) respectively at 5% conc. after 7days of seed treatment. In terms of inhibition rate, petroleum ether extract at 5% conc. was found to be most effective (77.29%), followed by methanol (76.65%), ethanol (74.78%) and water extract (74.42%) respectively from 1st day to 10th days of adult emergence.

Keywords: *Ocimum sanctum*, *Callosobruchus chinensis*, Mortality, Oviposition deterrence

1. Introduction
Insects are a problem in stored grain throughout the world. Pulse bruchids (*Callosobruchus chinensis*) are the most serious insect pests of storedpulses throughout the tropical countries. It is one of the most devastating insect pests of all pulses causing 40-50% losses of pulses in storage (Gosh and Durbey, 2003) [6]. It causes substantial loss and damageto seeds of many legumes especially green gram (*Vigna radiata*) which is major source ofdietary protein and other essential nutrients.In order to keep these stored grains free frompest attack, various synthetic pesticides have been used (Opolot *et al.,* 2006) [8]. Although they are effective, their repeated use for decade has disrupted natural biological control system and lead to outbreak of resistant pests to various types of insecticides, undesirable effects on non-target organism, environment and human health concern (Owens, 1986) [9]. Therefore environment needs some other alternative of chemical pesticides. Plants essential oils are alternative ofsynthetic pesticides possess insecticidal, ovicidal, repellent and ovipositional activities against various stored product insects (Chiasson *et al.,* 2004; Aboua *et al.,* 2010) [4]. Plant essential oilsare potential source of alternative compounds to currently use as contact orfumigant pesticides because they include a rich source of bioactive compounds.
Keeping this in view, the present investigation of insecticidal activity of *O. sanctum* was evaluated against *C. chinensis* on mortality, oviposition and hatchability in stored green gram seed.

2. Materials and Methods
2.1 Rearing of the Test Insect
The experiments were conducted at the Physiology Laboratory, Department of Entomology, Assam Agricultural University (AAU), Jorhat during the year 2014, October to April, 2015. Rearing of *C. chinensis* was maintained on green gram seed. For maintaining the culture of adult *C. chinensis*, 1kg green gram seed were put in a 5 lit capacity plastic jar and released five

pairs of adult male and female in 1:1 ratio. For proper growth and development of the insect during winter season, the plastic jar containing green gram seed and *C. chinensis* were kept on BOD incubator at temperature 29±2 °C.

2.2 Extraction of Bioactive Compounds

The leaves of *O. sanctum* were collected in and around Jorhat district of Assam, India.The collected leaves were washed and dried in the shade at room temperature, grounded finely and hydro distilled in a Soxhlet apparatus as well as extracted separately withmethanol, ethanol, and petroleum ether as per method described by Bora *et al*. (1999) [3].The solvent were removed under reduced pressure using rotary vacuum evaporator (JSGW) and the residues were further dissolved in respective solvents on weight by volume (W/V) basis making it 100% stock solutionand stored in a sealed glass bottle at 4ºC refrigerator. Similarly the aqueous extract were prepared grinding leaves in distilled water with weight by volume basis after washing thoroughly with running water which served as 100% stock solution.

2.3 DirectToxicity Test

The bioassays of *O. sanctum* on *C. chinensis* were performed by following the method of Talukdar and Howse (1993) [12] with some modifications. The adult insect were picked up from the stock culture and transferred to 9 cm diameter petriplates. Then 0.1ml solutions of different concentrations (1%, 1.5%, 2%, 2.5%, 3.5%, 4%, 4.5% and 5% W/V) were applied topically to the dorsal surface of the thorax of each insect by using hand atomizer (100ml). Released the treated insect immediately in the plastic container containing 20g green gram seeds. Insect mortality rates were recorded after 24hr, 48hr, 72hr and 96hr after treatment. Insect were examined daily and those that do not move or respond to gentle touch were considered dead. All the experiments were conducted CRD with five replication containing five pairs of insect in each replication and subjected to statistical analysis.

$$\text{Mortality \%} = \frac{\text{Total no. of mortality of Pulse beetle in treated plastic container}}{\text{Total no. of insect recorded in each plastic container}} \times 100$$

2.4 Total Residual Toxicity Test

Plant extracts were mixed with 20g green gram seed @ 1%, 1.5%, 2%, 2.5%, 3%, 3.5%, 4%, 4.5% and 5% W/V. The treated seeds were air dried for 20 min and then put in to separate plastic pot (6cm×7cm). Fresh insect were released in each plastic pot containing 20g treated seed and closed it immediately after released of the insect. The whole experiments were replicated 5 times with 5 pairs of insects.

2.5 Oviposition Deterrence Activity

Five pairs of newly emerged beetles were released in pot containing (6cm×7cm) 20g green gram seed treated with different concentrations of each plant extracts allowed to remain in container for 7 days till they lay eggs. After one week of oviposition the number of eggs laid on treated seed (Et) and control seed (Ec) were counted and the percentage of oviposition deterrence (POD) were calculated using the following formula

$$\text{POD (\%)} = \frac{\text{Ec - Et x100}}{\text{Ec}}$$

Where, Et = No. of eggs laid on treated seed
Ec = No. of egg laid on Control seed

2.6 Adult Emergence Test

Pulse beetle starts to emerge after 30-40 days of egg laying. Thee merge beetles were count and remove every day from the container. The numbers of beetles were count daily from the date of first emergence to at least 10 days. The emergence rate were calculated and the inhibition rate (IR %) were calculated using the following formula

$$\text{IR\%} = \frac{\text{Cn - Tn x100}}{\text{Cn}}$$

Where, C_n= Number of insects in control plastic pots
T_n = Number of insects in treated plastic pots

3. Statistical analysis

The experimental data in percentage were subjected to angular transformation before analysis. Zero per cent and 100 per cent values were substituted by 1/4n and (100-1/4n) respectively to correct the mortality percentage as suggested by Steel and Torrie (1960). The per cent mortality was corrected whenever we get 10-20 per cent mortality in the control treatment by using formula suggested by Abbot (1925).

$$\text{Corrected mortality} = \frac{\text{\% mortality in the treatment - \% mortality in control}}{\text{100 - \% mortality in the control}} \times 100$$

Statistical analysis of the angular transform data was done using Fisher's method of Analysis of Variance (ANOVA) with Completely Randomised Design (CRD).

The significant or non-significant difference between treatment mean were ascertained by Duncan's (1955) Multiple Range Test (DMRT) using a computer programme, SPSS (Version 20). The alphabetic notation was used to denote significant or non-significant difference among the treatment means. Mean lethal concentration (LC_{50}) are computed by following the probit analysis using a computer programme, SPSS (version 20.0).

4. Results and Discussion

The effect of leaf extracts of *O. sanctum* on the adult *C. chinensis* is presented in Table 1 and 2. It was evident from the Table 1 and 2 that all the treatments differs significantly (P=0.05) and were superior to control in regards of adult mortality. While studying the effect of leaf extracts of *Ocimum sanctum* on the adult mortality of *C. chinensis*, petroleum ether extract recorded the highest mortality (84%) of adults at 5.00 per cent concentration in 96 HAT, which was followed by methanol (82%); ethanol (80%) and water extract (76%). It was supported by the findings of Pugazhvendan *et al*. (2012) [10], who reported that maximum percentage of mortality 76% and 92% at 48hrs and 72hrs respectively in Tulsi oil against *Tribolium castaneum* beetles at 5 ml and 10 ml concentrations. The present investigation was also supported by Kiradoo and Srivastava (2011) [7], who suggested that the leaves of *Ocimum basilicum*, *O. sanctum* and *Mentha spicata* in the form of various formulations have a potential to be used against the pest *C. chinensis* and can be employed as an alternate to chemical insecticides in household and storehouse to minimize the infestation and damage caused by the bruchid.

The effects of different solvent extracts of *O. sanctum* on ovipositional responses of *C. chinensis* are given in Table 3.The effects of different solvent extracts of *O. sanctum* on ovipositional properties of *C. chinensis* were evaluated and

found that the highest oviposition deterrence (60.75%) in the seed treated with petroleum ether extract of *O. sanctum,* followed by methanol, ethanol and water extractrecorded 59.38%, 56.22% and 50.11%, respectively at 5.00% concentrations after 7 days of seed treatment. It was supported by the work of Ratnasekera and Rajapakse (2012) [11], who reported that the oils of *O. sanctum* at 1.5µL completely inhibited oviposition of *C. chinensis.*

The effect of different solvent extracts of *O. sanctum* on hatching success of *C. chinensis* eggs are given in Table 4. It shows that the ovicidal effect of the different solvent extracts of *O. sanctum,* where all the treatments were significantly different amongst themselves. In terms of hatching success of *C. chinensis* eggs after treated with different solvent extracts of *O. sanctum.* It was observed that petroleum ether extract of *O. sanctum* at 5.00% concentration was found to be most effective, inhibits the adult emergence up to 77.29%, followed by methanol (76.65%), ethanol (74.78%) and water extract (74.42%) respectively from 1 day to 10 day of adult emergence. It was supported by the work of Ratnasekera and Rajapakse (2012) [11], who reported that the oils of *O. sanctum* at 1.5µL completely inhibited adult emergence of *C. chinensis.*

5. Conclusion

The results of the study have confirmed that the pulse beetle, *C. chinensis*, can be effectively controlled by leaf extract of *O. sanctum*. It may be due to the presence of volatile oil, which is rich in Eugenol & Cryophyllene, methyl chavicol, cinnamate in the leaves of *O. sanctum*. The use of botanicals should be encouragedin small farm storage, as the cost of these botanicals is low andeasily available when compared with the losses incurred in untreated seeds. Thus, the present investigations indicate that botanical derivatives might be useful as insect control agents for commercial use.

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Table 1: Cumulative percentage mortality of adult *C. chinensis* treated with petroleum ether and methanol extracts of *Ocimum sanctum* leaf

Conc. (%)	Petroleum ether (Mean±SE)				Methanol (Mean ± SE)			
	24 HAT	48 HAT	72 HAT	96 HAT	24 HAT	48 HAT	72 HAT	96 HAT
1.00	6±2.45 (14.17)^{e}	16±2.45 (23.57)^{h}	20±0.00 (26.55)^{g}	24±2.45 (29.32)^{i}	6±2.45 (14.17)^{e}	14±2.45 (21.96)^{h}	18±2.00 (25.09)^{g}	22±2.00 (27.96)^{g}
1.50	12±3.74 (20.26)^{d}	22±2.00 (27.96)^{g}	26±3.99 (30.64)^{f}	30±4.46 (33.20)^{h}	10±4.46 (18.43)^{e}	18±2.00 (25.09)^{g}	24±3.99 (29.32)^{f}	28±3.74 (31.94)^{f}
2.00	18±3.74 (25.09)^{c}	26±5.09 (30.64)^{f}	34±5.09 (35.65)^{e}	40±4.46 (39.22)^{g}	16±3.99 (23.57)^{d}	24±3.99 (29.32)^{f}	30±3.16 (33.20)^{f}	36±3.99 (36.86)^{e}
2.50	20±3.16 (26.55)^{c}	34±3.99 (35.65)^{e}	42±3.74 (40.38)^{d}	46±3.99 (42.69)^{f}	20±3.16 (26.55)^{c}	36±2.45 (36.86)^{e}	42±3.74 (40.38)^{e}	48±2.00 (43.84)^{d}
3.00	22±3.74 (27.96)^{c}	40±0.00 (39.22)^{d}	50±3.16 (44.98)^{c}	54±2.45 (47.28)^{e}	22±3.74 (27.96)^{c}	40±3.16 (39.22)^{de}	48±3.74 (43.84)^{d}	52±4.89 (46.13)^{c}
3.50	26±2.45 (30.64)^{bc}	44±5.09 (41.54)^{cd}	52±6.62 (46.13)^{bc}	60±4.46 (50.75)^{d}	26±2.45 (30.64)^{b}	42±7.34 (40.38)^{cd}	50±5.47 (44.98)^{d}	56±3.99 (48.43)^{c}
4.00	30±3.16 (33.20)^{bc}	48±2.00 (43.84)^{bc}	58±5.82 (49.58)^{b}	64±5.09 (53.11)^{c}	28±3.74 (31.94)^{ab}	46±5.09 (42.69)^{c}	54±5.09 (47.28)^{c}	58±3.74 (49.58)^{bc}
4.50	32±3.74 (34.44)^{ab}	50±4.46 (44.98)^{b}	60±3.16 (50.75)^{b}	76±2.45 (60.64)^{b}	32±3.74 (34.44)^{a}	50±4.46 (44.98)^{b}	60±6.31 (50.75)^{b}	70±5.47 (56.77)^{b}
5.00	36±2.45 (36.86)^{a}	60±4.46 (50.75)^{a}	78±3.74 (62.00)^{a}	84±2.45 (66.40)^{a}	34±5.09 (35.65)^{a}	56±5.99 (48.43)^{a}	72±6.62 (58.03)^{a}	82±3.74 (64.87)^{a}
Control	0±0.00 (1.28)^{f}	0±0.00 (1.28)^{i}	4±2.45 (11.53)^{h}	6±2.45 (14.17)^{j}	0±0.00 (1.28)^{f}	2±2.00 (8.13)^{i}	4±2.45 (11.53)^{h}	4±2.45 (11.53)^{h}
S. Ed (±)	1.26	0.98	1.29	1.17	1.43	1.28	1.39	1.20
CD (P=0.05)	2.06	1.61	2.12	1.92	2.34	2.10	2.28	1.97

*Data presented are the mean of 5 replications each having 10 nos. of insects.
* Zero and 100% mortality was corrected by using Steel & Torrie formula.
* Data within the parentheses are angular transformed value, compared by DMRT, (P<0.05)
* Means followed by same letter are not significantly different
* HAT= Hours after treatment

Table 2: Cumulative percentage mortality of adult *C. chinensis* treated with ethanol and water extracts of *Ocimum sanctum* leaf

Conc.(%)	Ethanol (Mean ± SE)				Water (Mean ± SE)			
	24 HAT	48 HAT	72 HAT	96 HAT	24 HAT	48 HAT	72 HAT	96 HAT
1.00	6±2.45 (14.17)^{h}	14±2.45 (21.96)^{g}	20±3.16 (26.55)^{g}	22±3.74 (27.96)^{h}	4±2.45 (11.53)^{g}	12±3.74 (20.26)^{g}	16±2.45 (23.57)^{h}	20±3.16 (26.55)^{i}
1.50	14±2.45 (21.96)^{g}	24±6.77 (29.32)^{f}	32±6.62 (34.44)^{f}	34±5.09 (35.65)^{g}	12±3.74 (20.26)^{f}	22±6.62 (27.96)^{f}	28±3.74 (31.94)^{g}	30±3.16 (33.20)^{h}
2.00	20±4.46 (26.55)^{f}	34±5.09 (35.65)^{e}	40±3.16 (39.22)^{e}	44±2.45 41.54)^{f}	18±2.00 (25.09)^{e}	30±3.16 (33.20)^{e}	32±2.00 (34.44)^{f}	40±3.16 (39.22)^{g}
2.50	24±2.45 (29.32)^{e}	42±2.00 (40.38)^{d}	46±2.45 (42.69)^{e}	52±2.00 (46.13)^{e}	22±2.00 (27.96)^{de}	32±3.74 (34.44)^{de}	42±4.89 (40.38)^{e}	48±2.00 (43.84)^{f}
3.00	26±5.09 (30.64)^{de}	44±2.45 (41.54)^{cd}	54±2.45 (47.28)^{d}	62±2.00 (51.92)^{d}	24±5.09 (29.32)^{cd}	36±2.45 (36.86)^{d}	50±4.46 (44.98)^{d}	56±5.99 (48.43)^{e}
3.50	28±3.74 (31.94)^{cd}	48±5.82 (43.84)^{bc}	58±5.82 (49.58)^{cd}	68±3.74 (55.53)^{c}	26±3.99 (30.64)^{bc}	40±5.47 (39.22)^{cd}	54±3.99 (47.28)^{c}	60±3.16 (50.75)^{d}
4.00	30±3.16 (33.20)^{bc}	50±4.46 (44.98)^{ab}	62±7.34 (51.92)^{c}	70±5.47 (56.77)^{c}	30±3.16 (33.20)^{ab}	46±5.09 (42.69)^{c}	56±7.47 (48.43)^{c}	64±5.09 (53.11)^{c}
4.50	32±3.74 (34.44)^{ab}	54±3.99 (47.28)^{a}	68±3.74 (55.53)^{b}	76±5.99 (60.64)^{b}	32±3.74 (34.44)^{a}	50±5.47 (44.98)^{b}	62±4.89 (51.92)^{b}	68±5.82 (55.53)^{b}
5.00	34±2.45 (35.65)^{a}	56±2.45 (48.43)^{a}	72±6.62 (58.03)^{a}	80±4.46 (63.41)^{a}	34±3.99 (35.65)^{a}	56±3.99 (48.43)^{a}	70±4.46 (56.77)^{a}	76±3.99 (60.64)^{a}
Control	2±2.00 (8.13)^{i}	2±2.00 (8.13)^{h}	4±2.45 (11.53)^{h}	8±2.00 (16.42)^{i}	0±0.00 (1.28)^{d}	0±0.00 (1.28)^{h}	2±2.00 (8.13)^{f}	4±2.45 (11.53)^{j}
S. Ed (±)	1.28	1.25	1.47	1.28	1.29	1.52	1.28	1.30
CD (P=0.05)	2.09	2.05	2.42	2.09	2.11	2.50	2.11	2.14

*Data presented are the mean of 5 replications each having 10 nos. of insects.
* Zero and 100% mortality was corrected by using Steel &Torrie formula.
* Data within the parentheses are angular transformed value, compared by DMRT, (P<0.05)
* Means followed by same letter are not significantly different
* HAT= Hours after treatment

Table 3: Ovipositional response of *C. chinensis* female to different extracts of *Ocimum sanctum* on treated green gram seed

Conc. (%)	Petroleum ether		Methanol		Ethanol		Water	
	No of eggs/20g seeds (Mean ± SE)	Oviposition deterrence %	No of eggs/20g seeds (Mean ± SE)	Oviposition deterrence %	No of eggs/20g seeds (Mean ± SE)	Oviposition deterrence %	No of eggs/20g seeds (Mean ± SE)	Oviposition deterrence %
1.00	93.40±3.55^{b}	24.79	98.20±3.94^{b}	22.97	92.00±5.91^{b}	18.28	96.20±3.03^{b}	18.90
1.50	90.00±2.73^{c}	27.33	94.00±3.67^{c}	26.16	87.60±5.49^{c}	22.22	90.20±3.43^{c}	23.97
2.00	84.80±4.20^{d}	31.76	89.00±5.05^{d}	30.70	85.80±5.16^{cd}	23.91	89.00±4.93^{c}	24.95
2.50	80.20±3.42^{e}	35.04	82.80±4.41^{e}	35.09	83.80±3.79^{d}	25.96	88.20±5.47^{c}	25.16
3.00	73.60±3.38^{f}	40.26	76.40±5.04^{f}	39.41	83.40±6.83^{d}	26.05	88.00±5.06^{c}	25.62
3.50	69.40±3.41^{g}	44.12	71.40±4.60^{g}	44.46	77.80±4.50^{e}	30.65	80.60±3.76^{d}	31.89
4.00	60.00±3.41^{h}	51.47	65.20±4.68^{h}	48.99	70.00±3.27^{f}	37.92	75.60±5.00^{e}	35.95
4.50	53.60±5.90^{i}	56.47	57.60±6.90^{i}	54.45	60.20±2.55^{g}	46.65	68.00±4.48^{f}	42.35
5.00	48.60±3.30^{j}	60.75	51.20±4.70^{j}	59.38	49.40±6.13^{h}	56.22	58.80±4.30^{g}	50.11
Control	124.80±5.41^{a}	0.00	128.80±6.13^{a}	0.00	113.40±4.03^{a}	0.00	118.60±3.27^{a}	0.00
S. Ed (±)	1.79	1.74	2.24	2.16	2.21	2.29	1.95	1.74
CD (P=0.05)	2.93	2.86	3.67	3.54	3.63	3.75	3.20	2.85

*Data presented are the mean of 5 replications each having 10 nos. of insects.
* 20 g seed content approx. 560-565 seeds
* The mean values were compared by DMRT, (P<0.05)
* Means followed by same letter are not significantly different.

Table 4: Effect of *Ocimum sanctum* leaf & flower extracts on hatching success (%) of *C. chinensis* eggs on treated green gram seed

Conc. (%)	Petroleum ether		Methanol		Ethanol		Water	
	No of insect emergence (% hatching) (Mean ± SE)	Hatching inhibition rate over control	No of insect emergence (% hatching) (Mean ± SE)	Hatching inhibition rate over control	No of insect emergence (% hatching) (Mean ± SE)	Hatching inhibition rate over control	No of insect emergence (% hatching) (Mean ± SE)	Hatching inhibition rate over control
1.00	39.20±0.86 (38.75)^{b}	24.51	38.60±0.60 (38.39)^{b}	23.85	39.40±0.75 (38.86)^{b}	22.51	39.40±0.75 (38.86)^{b}	21.51
1.50	35.40±2.06 (36.50)^{c}	31.98	35.20±1.53 (36.38)^{c}	30.59	35.60±1.69 (36.62)^{c}	29.97	35.60±1.69 (36.62)^{c}	29.21
2.00	33.00±2.12 (35.05)^{cd}	36.47	31.60±1.91 (34.19)^{d}	36.81	33.00±1.41 (35.05)^{c}	35.38	33.00±1.41 (35.05)^{c}	34.51
2.50	30.80±1.32 (33.70)^{d}	40.69	30.60±2.97 (33.57)^{de}	39.29	31.00±1.64 (33.82)^{d}	39.32	31.00±1.64 (33.82)^{d}	38.53
3.00	28.40±1.36 (32.19)^{de}	45.32	28.40±2.06 (32.19)^{e}	43.24	29.40±2.04 (32.82)^{de}	42.56	29.40±2.04 (32.82)^{de}	41.69
3.50	25.60±2.61 (30.38)^{e}	50.38	25.40±2.73 (30.25)^{f}	49.79	26.60±2.33 (31.04)^{e}	48.01	26.60±2.33 (31.04)^{e}	47.19
4.00	18.20±1.43 (25.24)^{f}	65.02	17.60±1.36 (24.79)^{g}	65.62	19.00±1.64 (25.83)^{f}	63.20	19.00±1.64 (25.83)^{f}	62.61
4.50	13.60±0.93 (21.63)^{g}	73.74	14.00±0.71 (21.96)^{h}	72.24	15.80±1.02 (23.41)^{g}	68.94	15.80±1.02 (23.41)^{g}	68.53
5.00	11.80±0.91 (20.08)^{g}	77.29	11.60±0.75 (19.90)^{i}	76.65	12.80±0.86 (20.95)^{h}	74.78	12.80±0.86 (20.95)^{h}	74.42
Control	52.00±1.30 (46.13)^{a}	0.00	51.60±3.64 (45.90)^{a}	0.00	51.40±2.27 (45.78)^{a}	0.00	50.60±1.77 (45.33)^{a}	0.00
S. Ed (±)	0.47	1.35	0.58	2.42	0.48	1.63	0.47	1.57
CD (P=0.05)	0.77	2.22	0.95	3.97	0.79	2.68	0.77	2.57

*Data presented are the mean of 5 replications each having 10 nos. of insects.

* Data within the parentheses are angular transformed value, compared by DMRT, (P<0.05)

* Means followed by same letter are not significantly different

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