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Effects of *Citrus sinensis* Peel Oil on the Oviposition and Development of Cowpea Beetle *Callosobruchus maculatus* (Coleoptera: Chrysomelidae) in Some Legume Grains

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Abstract.- The present study was undertaken to study the effect of *Citrus sinensis* peel oil on the oviposition and development of *Callosobruchus maculatus* in some legume grains. Oviposition deterrency and F_1 adult emergence were carried out with split plot design of six concentrations (0.25, 0.5, 0.75, 1.0, 1.5, 2.0 ml) replicated four times on cowpea, bambara groundnut and pigeon pea against *C. maculatus.* The phytochemical study of *C. sinensis* peel oil revealed that ß – pinene and limonene were the most active ingredients with percentage chemical contents of 9.3 and 70 respectively. The mean developmental periods of *C. maculatus* in the selected legumes showed significant differences while the percentage emergence of beetle was highest in cowpea (95%) followed by bambara groundnut (84%) and pigeon pea (68%). The sex ratio of emerged *C. maculatus* in the three legumes showed that more females than males were emerged, and emergence (%) was higher in cowpea than other legumes. The mean fecundity in the three legumes showed that *C. maculatus* was most fecund (137.8±23.44) in cowpea, followed by bambara groundnut (131.2±34.24) and pigeon pea (99.0±24.4). Maximum deterrent activity in oviposition and adult emergence was observed at 1.5 to 2.0 ml peel oil treatment in all the grain types. Based on this study *C. sinensis* peel oil could be suggested for use to suppress populations of *C. maculatus* for short to moderate periods of storage.

Key words: Oviposition, longevity, *Citrus sinensis* peel oil, *Callosobruchus maculatus*, legumes.

INTRODUCTION

Globally a minimum of 10% of the cereals and legumes are lost after harvest each year due to insect pests (Dike and Mshelia, 1997). Insect pests cause heavy economic losses to stored grains throughout the world and their impacts are more devastating in developing countries. The most universal pest in pulse storage is the cowpea beetle *Callosobruchus maculates*. They cause losses of about 30% of stored beans (Permonge *et al*., 1997). Its oviposition and growth are continuous and the larva feeds on the seeds. After emergence from the seeds, the adults reproduce either in the stored seeds in a continuous cycle or in the field where the grains are planted.

The control of the stored products insects relied heavily on the hazardous synthetic products. The increasing problems associated with the use of synthetic chemicals for the control of stored products insects necessitates the development of safe strategies like natural agents. In this regard the use of plant products as bio-insecticides is one of the important approaches to insect management and has many advantages over synthetic insecticides (Weinzierl and Henn, 1992). Plant materials with insecticidal properties are the most important locally available method for the biological control of pest and provide inexpensive method for the small-scale farmers with locally available biodegradable and for the protection of stored products.

Citrus is one of the most important commercial fruit crops grown in all continents of the world (Tao *et al*., 2008). Citrus importance is attributed to its diversified use and growing world demand with about 102.64 million tones total world production and probably stands first largest among the produced fruit (Manthey and Grohmann, 2001). By-product recovery from fruit wastes can improve the overall economics of processing units. Besides this, the problem of environmental pollution also can be reduced considerably. The citrus peels are rich in nutrients and contain many phytochemicals; they can be efficiently used as drugs or as food supplements too (Nand, 1998). Cowpeas treated with the orange peel oil and powder have been

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reported to deter *Callosobruchus maculatus* feeding, resulting to poor development and death (Don Pedro, 1985). The orange peel oil was reported to be toxic toward *Culex pipiens* and cowpea beetles (Don Pedro, 1985).Considering the importance of bio-insecticide in the pest management, the present work was designed to investigate the efficacy of orange peel oil in deterring the oviposition and interference in the developmental stages of C*allosobruchus maculatus* (Islam *et al*., 2007) in different grains. The leguminous grains selected in this study were based on people's wide acceptance or choice and use for human food, animal feed and in confectionary industries.

MATERIALS AND METHODS

Legumes

The cowpea beetle, *C. maculatus* was reared on selected legumes *viz.*, cowpea, IT 89KD-288 (now SAMPEA- 11, large white seed and rough seed coat), bambara groundnut; B1, large dark red seed and rough seed coat and pigeon pea: 5C, small light brown seed colour and smooth coat collected from the local market.

Infestation of legumes with beetle

Each culture contained 500 g of each of the selected legumes infested initially with insects obtained from pure culture maintained at Entomology Research Laboratory. All culture jars and selected legumes were dry heated for 2 h before commencement of experiment. All cultures and experimental setups were maintained at room temperature of 28±2°C and 56-65% relative humidity (r.h). All culture jars were covered with muslin cloth, and fastened with rubber band to resist insects from escaping the jars and also prevent exogenous insects from entering the jars.

Rearing of C. maculatus *eggs*

C. maculatus was allowed to lay eggs on each of the selected grains. When eggs were noticed on the grains, the insects were sieved out with a 2 mm mesh sieve and the legumes with eggs were allowed to develop.

Egg hatch

Eggs were noticed on the grains on the sixth day, eggs laid on the legumes hatched inside the seeds within ten days at room temperature (28-30°C) and r.h. 45-65%. The hatching of eggs was monitored daily (Oparaeke, 1996). Presence of black head inside the seeds indicated the presence of larvae and percentage egg hatch was calculated based on the number of black head per grain.

Extraction of oil from C. sinensis *peel*

One thousand gram (1000 g) of *C. sinensis* peels were sun dried for 6 days until constant weight was obtained. The dried peels were powdered using electric blender and 400 g of the powdered plant material was soaked in 800 ml distilled N-hexane at room temperature for 24 hours using cold pressing through decanting method at 130 rpm. The extract was collected in a collecting flask and filtered, using Whatman filter paper. The extract was transferred to glass jar with cover and kept in a refrigerator until needed.

Phytochemical and chemical composition of C. sinensis *peel oil*

The powdered plant part as well as the extract was subjected to phytochemical and chemical screening following methods of Harborne (1973), Sofowora (1993) and Douglas *et al*. (1996)**.**

For chemical extraction 2.0 g of sweet orange citrus peel was used. One should be absolutely certain to make sure that the rind is grated into very fine particles for which the smallest texture for the grating process be used and special care to be taken not to damage the pulp of the fruit, since most of the essential oils are found in the sacks within the peel of the fruit, not in the pulp. This is done to maximize the surface area for the extraction process.

The citrus rind (ca 2.0 g) was added to the separatory funnel along with 7.0 ml of pentane. The mixture was extracted for approximately ten minutes. The separatory funnel should be frequently vented as pentane is very volatile. The pentane solution was drained into a 50 ml Erlenmeyer flask. This procedure was repeated two more times using two additional 7.0 ml aliquots of pentane. All the pentane solution was collected together and then approximately 1.0 g of sodium sulfate (Na_2SO_4) was added to the pentane solution. The Erlenmeyer flask was covered and the mixture allowed to stand for approximately ten minutes with occasional swirling.

After the solution had dried sufficiently (it no longer appears cloudy), the pentane solution was carefully decanted into a pre weighed 60 ml beaker, and the pentane was evaporated in a sand bath using low heat and a gentle stream of air over the mouth of the beaker in the fume hood. Once the evaporation is complete, mass of the crude citrus fruit extract was recorded. It appeared as viscous amber oil, diluted with approximately 1.0 ml of dichloromethane (methylene chloride) and the resulting solution transferred into a 3.0 ml conical reaction vial. Finally, approximately 0.25 µl of the dichloromethane solution was injected into the GC/MS and the data was analysed.

Developmental period, sex ratio and adult longevity

The developmental period of *C. maculatus* was studied on cowpea, bambara groundnut and pigeon using a split plot design of seven treatments per (0, 0.25, 0.5, 0.75, 1.0, 1.5 and 2.0 ml) pea legume type replicated four times. Forty grams of each legume were introduced into 72 glass jars (diameter = 0.09 m, volume = 3.69 m^3). Two pairs of newly emerged adults (0-1 day old) were introduced in 40 g of treated legumes of each type. Each jar was covered with a muslin cloth held in place by a rubber band to allow air movement and was left undisturbed until oviposition occurred. Daily oviposition by *C. maculatus* was monitored from the onset of oviposition to death of the introduced adults. The highest lasted egg laying adult stayed for 12 days and counting of eggs stopped. Egg hatching, *C. maculatus* developmental stages, sex ratios, adult longevity of *C. maculatus* were monitored and recorded. The experiments were replicated four times.

Fecundity

Pairs of newly emerged (0-1 day old) males and females *C. maculatus* introduced into 40 g each of the selected legume grains treated with each concentration in glass jars covered with muslin cloth. Each treatment was replicated five times. The eggs laid by each female beetle were counted daily till the death of the female beetle. The mean fecundity of the beetles on the selected legumes was determined.

Data analysis

Data were analysed by analysis of variance to establish the means, while significant differences were compared by New Duncan Multiple Range Test (NDMRT) ($P<0.05$) using SPSS statistical software package.

RESULTS

Phytochemical analysis of Citrus sinensis *peel oil*

The chemical constituents of the essential oil of *C. sinensis*, the retention indices and the percentage of the individual components are summarized in Table I. The two major active ingredients were β-pinene (9.3%) and limonene (70%).

Table I.- Phytochemical components of the essential oil of *Citrus sinensis* peel.

Compounds	Retention index	Content (%)
ß-pinene	973	9.3
Limonene	1040	70
Linalool	1104	3.8
Isopulegol	1156	0.56
Terpinen-4-ol	1185	0.59
Decanal	1208	4.92
Geranyl formate	1384	1.92
Citral	1159	0.89
Helminthogermacrene	1382	0.5
1,1-dodecanediol	1452	0.94
Δ-murrolene	1480	1.58
Valencene	1495	3.81

Egg hatchability

The egg hatchability was 95% for cowpea, 84% for bambara groundnut and 68% for pigeon pea (Table II). The percentage hatchability of *C. maculatus* eggs was highest in cowpea. There were significant differences in the developmental period (days) of *C. maculatus* which emerged from the treated legumes (Table III). The higher the concentrations the more prolonged the developmental time. The adult longevity of males was 3.15±0.01 days in pigeon pea, 4.43±0.86 in cowpea while the longest male was observed in bambara groundnut 7.39±1.63 days (Table IV). The adult longevity of females ranged from 4.25±0.06 in

Table II.- **Percentage egg hatchability of *C. maculatus* reared on different legumes at 28-30°C, 50-65% r.h**

Legume type	Mean hatchability period ± SE (days)	n	% emergence of beetle
Cowpea	34.80 ± 0.06[a] (28-34)	285	95
Bambara groundnut	32.74 ±0.05[b] (26-32)	252	84
Pigeon pea	31.08 ± 0.03[b] (28-35)	204	68

Means in the same vertical column followed by the same letter do not differ significantly at 5% level probability.

pigeon pea, 10.30±1.90 in cowpea and 12.15±2.05 days in bambara groundnut. The sex ratio of *C. maculatus* that emerged on the different legume seeds showed that more ($p>0.05$) male beetles emerged in bambara groundnut (54.7%) followed by pigeon pea (48.9%) and the least in cowpea (30.6%), while more ($p<0.05$) females emerged in cowpea (79.8%), followed by bambara groundnut (69.8%) and the least in pigeon pea (49.2%) (Table V).

Considering the total emergence of *C. maculatus* in the legumes, the sex ratio (♂♀) of *C. maculatus* ranged from 1: 2.2 in cowpea, followed by 1:1.5 in Bambara groundnut and 1:1.1 in pigeon pea (Table V).The mean fecundity of *C. maculatus* on the treated legumes showed that *C. maculatus* was most fecund on cowpea seed at the rate of 137.8±23.44, followed by Bambara groundnut at the rate of 131.2± 34.24 and pigeon pea at the rate of 99.0±24.41 (Table VI).

Efficacy of C. sinensis *peel oil against* C. maculatus

Adult emergence of *C. maculatus* was recorded highest on untreated legumes. The action of *C. sinensis* peel oil was least on treated legumes with dosage of 0.25 ml per 40 g of legume seed. Highest dose (2.0 ml) of *C. sinensis* suppressed *C. maculatus* emergence by almost 100 % (Table VII).

DISCUSSION

The present study concludes that ß – pinene and limonene were the main active ingredients in *Citrus sinensis* which interfered with the physiology of *Callosobruchus maculatus.* Taylor (1975) reported that sweet orange and grape peel oils interfere with the physiology of insect pest *C. maculatus*. The percentage hatchability of *C. maculatus* eggs was highest in cowpea. This could be attributed to the fact that cowpea is a preferred host for egg laying and development by *C. maculatus*. Creadland *et al*. (1986) and Van-Hius and de Rooy (1998) also reported that oviposition rate of *C. maculatus* was maximum in cowpea. Jayakumar (2010) recorded egg hatchability of *C. maculatus* in cowpea seeds was 64% and 62 % when treated with *Cassia auriculata* (Linn,1753) and *Artemisia nilagirica* (Linn,1753), respectively. Islam *et al*. (2007) recorded maximum hatching of *C. maculatus* eggs in black gram (92.18%) followed by Bengal gram (75.5%), green peas (74.24%) and lentil (54.59%). Cox *et al.* (1981) observed that egg hatching of the beetle was less at 20 % r.h, than at 50 % r.h.

There were significant differences in the developmental period (days) of *C. maculatus* which emerged from the treated legumes showing that *C. sinensis* peel oil delayed developmental period of the beetle compared to the control. The higher the concentration the more prolonged the developmental time. Righi-assia *et al.* (2010) observed that oils and powders of some plants like *Thymus vulgarus* (Linn, 1753), *Santolina chaecyparissus* (Linn, 1753) and *Anagyris foetida* (Lineo, 1753) showed effectiveness in delaying emergence of *C. maculatus*.

There were significant differences in the adult longevities of *C. maculatus* ($P<0.05$) which emerged from the treated legumes. The adult longevity were comparable to those observed by Islam *et al*. (2007), who recorded a female longevity of 15.05 days and a male lifespan of 13.25 days. Cox *et al*. (1981) reported that unmated males live on average 5 days longer than females.

It is revealed from the present result that *C. maculatus* has different preference for different pulse seeds. Chemical cues and /or textures of the seed coat might be the reason for such differential choices. Teotia and Singh (1996), Shazali (1989) and Begum *et al.* (1993) published similar reports. There was no significant difference ($p > 0.05$) in the emergence of male beetles in different legumes, but significant differences ($p < 0.05$) was observed in the emergence of female beetle in the three legumes.

Table III.- Effect of different quantities of citrus peel oil (ml) on the duration of various developmental stages (in days) of *Callosobruchus maculatus* reared in different legumes at 28-30°C, 50-65% r.h.

Legume	Dosages of treatment peel oil (ml)						
	0.0	0.25	0.5	0.75	1.0	1.5	2.0
Egg stage							
Cowpea	6.00±0.00^{f1}	13.00±0.00^{e1}	13.00±0.00^{e1}	14.00±0.00^{d1}	15.00±0.00^{c1}	16.00±0.00^{b1}	18.00±0.00^{a1}
Bambara groundnut	5.00±0.00^{f2}	10.00±0.00^{e2}	10.00±0.00^{e2}	11.00±0.00^{d2}	12.00±0.00^{c2}	14.00±0.00^{b2}	16.00±0.00^{a2}
Pigeon pea	5.00±0.00^{f2}	9.00±0.00^{e3}	9.00±0.00^{e3}	10.00±0.00^{d3}	11.00±0.00^{c3}	13.00±0.00^{b3}	15.00±0.00^{a3}
Larva stage							
Cowpea	12.00±0.00^{f1}	19.00±0.00^{e1}	19.00±0.00^{e1}	20.00±0.00^{d1}	21.00±0.00^{c1}	22.00±0.00^{b1}	24.00±0.00^{a1}
Bambara groundnut	12.00±0.00^{f1}	16.00±0.00^{e2}	16.00±0.00^{e2}	17.00±0.00^{d2}	18.00±0.00^{c2}	20.00±0.00^{b2}	22.00±0.00^{a2}
Pigeon pea	12.00±0.00^{f1}	13.00±0.00^{e3}	13.00±0.00^{e3}	14.00±0.00^{d3}	15.00±0.00^{c3}	17.00±0.00^{b3}	19.00±0.00^{a3}
Pupa stage							
Cowpea	25.00±0.00^{f1}	33.00±0.00^{e1}	33.00±0.00^{d1}	34.00±0.00^{c1}	35.00±0.00^{b1}	36.00±0.00^{a1}	36.00±0.00^{a1}
Bambara groundnut	25.00±0.00^{f1}	30.00±0.00^{e2}	30.00±0.00^{e2}	31.00±0.00^{d2}	32.00±0.00^{c2}	33.00±0.00^{b2}	34.00±0.00^{a2}
Pigeon pea	25.00±0.00^{f1}	30.00±0.00^{e2}	30.00±0.00^{e2}	31.00±0.00^{d2}	32.00±0.00^{c2}	33.00±0.00^{b2}	34.00±0.00^{a2}
Adult stage							
Cowpea	27.00±0.00^{g1}	32.00±0.00^{f1}	33.00±0.00^{e1}	34.00±0.00^{d1}	36.00±0.00^{c1}	37.00±0.00^{b1}	38.00±0.00^{a1}
Bambara groundnut	27.00±0.00^{f1}	32.00±0.00^{e1}	32.00±0.00^{e2}	33.00±0.00^{d2}	34.00±0.00^{c2}	35.00±0.00^{b2}	37.00±0.00^{a2}
Pigeon pea	27.00±0.00^{f1}	31.00±0.00^{e2}	31.00±0.00^{e3}	32.00±0.00^{d3}	33.00±0.00^{c3}	34.00±0.00^{b3}	36.00±0.00^{a3}

In the different *Callosobruchus maculatus* developmental stages, means in the same row followed by the same letter do not differ significantly at 5% level and means in the same column followed by the same figure do not differ significantly at 5% level.

Table IV.- Summary of adult longevity of emerged *C. maculatus* reared on different legumes at 28-32°C, 54-65% r.h.

Legume	Mean adult longevity ± SE (days)	
	♂♂ (Male)	♀♀ (Female)
Cowpea	4.43±0.86^{a} (6-12)	10.30±1.90^{b} (8-14)
Bambara groundnut	7.39±1.6^{b} (4-12)	12.15±2.05^{c} (6-12)
Pigeon pea	3.15±0.01^{a} (3-10)	4.25±0.06^{a} (3-10)

Figure in parenthesis shows longevity ranges. Means in the same vertical column followed by the same letter do not differ significantly at 5% level probability.

There was a significant difference in the action of *C. sinensis* in the treatment groups. Considering the total emergence of *C. maculatus* in the legumes, more females emerged than males. Allotey (1991) recorded a sex ratio (♂♀) of approx 1:1 on maize and 1.5:1 on groundnut. The mean fecundity of *C. maculatus* on the treated legumes showed that *C. maculatus* was most fecund on cowpea seeds. No significant difference in the number of beetles that emerged in the legumes was found. Mannan and Bhuiyah (1996) reported that fecundity of *C. maculatus* in cowpea was over 85%, *i.e.* (186.15±0.17 in cowpea, 161.00±0.25 in Bengal gram and 130.67±0.67 in green peas). Allotey and Azalekor (1999) reared *Corcyra cephalonica* on groundnut, cowpea and Bambara groundnut and found that they laid more eggs on groundnut than on cowpea and Bambara groundnut, this could be attributed to *C. cephalonica* having groundnut as a preferred host.

Adult emergence of *C. maculatus* was recorded highest on untreated legumes. The action of *C. sinensis* peel oil was least on treated legumes with the highest dosage. Allotey and Azalekor (1999) reported that at a dose of 2.5 g, *C. sinensis*

Table V.- Effect of *Citrus sinensis* concentrations on the sex ratio of *Callosobruchus maculatus* in some leg.

Sex	Legumes	Concentrations (mls)							Summary of sex ratio (%)	Total emergence (♂♀)	Sex ratio (♂♀)
		0	0.25	0.5	0.75	1	1.5	2			
Male(♂)	Cowpea	2.70±0.86^{a2}	2.20±0.67^{b2}	1.76±0.56^{c2}	1.32±0.29^{d1}	1.02±0.06^{e1}	0.58±0.02^{f3}	0.29±0.01^{g2}	30.6%^{c}	222 *	01:02.2
	Bambara groundnut	2.52±0.42^{a3}	2.12±0.22^{b1}	1.81±0.19^{c1}	1.51±0.09^{d2}	1.01±0.08^{e1}	0.60±0.04^{f2}	0.40±0.02^{g1}	54.7%^{a}	219 *	01:01.5
	Pigeon pea	2.92±0.31^{a1}	2..02±0.28^{b2}	1.79±0.11^{c2}	1.12±0.08^{d3}	0.81±0.06^{e2}	0.78±0.05^{e1}	0.44±0.02^{f1}	48.9%^{b}	182 *	01:01.1
Female (♀)	Cowpea	2.72±0.21^{a3}	2.46±0.19^{b2}	1.94±0.12^{c2}	1.42±0.09^{d1}	0.77±0.07^{e3}	0.51±0.05^{f1}	0.12±0.01^{g3}	69.3%^{b}		
	Bambara groundnut	3.33±0.67^{a1}	2.83±0.38^{b1}	1.25±0.19^{c1}	1.08±0.10^{d3}	0.83±0.08^{e2}	0.50±0.06^{f1}	0.16±0.01^{g2}	79.8%^{a}		
	Pigeon pea	3.11±0.32^{a2}	2.04±0.21^{b3}	1.72±0.15^{c3}	1.29±0.10^{d2}	1.07±0.08^{e1}	0.53±0.06^{f1}	0.21±0.01^{g1}	49.2%^{c}		

*Total emergence for Male and Female (♂♀) *Callosobruchus maculates.*
In the different sex *Callosobruchus maculatus*, means in the same row followed by the same letter do not differ significantly at 5% level (for the concentration effect and summary of sex ratio) and means in the same column followed by the same figure do not differ significantly at 5% level (effect of concentration only)

powder suppressed moth emergence more effectively. Annie bright *et al*. (2001) and Raja *et al*. (2001) reported that botanicals inhibited adult emergence of *C. maculatus* in cowpea. The authors stated that when the beetle laid eggs on treated seeds, the toxic substances present in the extract may have entered into the eggs through chorion and suppress further embryonic development. Earlier literature indicate the importance of plant parts in protecting seeds by way of direct mixing of the dried leaves, plant powders, solvent extracts, vegetable / essential plant oils on seeds during post harvest storage (Rajapakse, 1996; Ngamo *et al.*, 2007; Meera and Lalitha, 2007; Zahra-sahaf and Moharramipour, 2008; Othira *et al.*, 2009). Jayakumar (2003) reported that plant extract have obvious effects on post embryonic survival of the insect resulting to reduction in adult emergence. The present work agreed with these submissions. Mixture of cowpea with dried powdered citrus peel has been found to reduce infestation and damage caused by *C. maculatus* (Taylor, 1975; Don Pedro, 1985). Other plant products that have been known with success both in powder and oil extract against insect infestation like *Azadiracta indica*, *Zingiber officinale*, *Allium sativum* etc. In the present study, *C. maculatus* at 1.5 and 2.0 mls / 40 g legume grain reduced beetle emergence by almost 100 %. Based on the present findings, extract of *C. maculatus* peel oil could be used as one of the components in integrated pest management especially in short time storage by farmers or shop retailers. The safety of the biopesticide against chemical pesticide (which is environmentally not friendly) is a factor that should be embraced and its awareness created amongst farmers and stored product retailers. *C. sinensis* peel oil is thus recommended for use to suppress population of *C. maculatus* for short to moderate periods of storage.

Table VI.- Summary of mean fecundity of *C. maculatus* reared on different legumes.

Legume type	Mean fecundity ± SE (range)
Cowpea	137.8 ± 23.44 (90-180)
Bambara groundnut	131.2 ± 34.24 (95-174)
Pigeon pea	99.0 ± 24.4 (86-162)

Table VII.- Effect of different doses of citrus peel oil on adult emergence of *C. maculatus* from selected legume grains treated with *C. sinensis* peel oil. The values in parentheses are % number of *C. maculatus* that emerged in different concentrations.

Legume	Dose of citrus peel oil (mls)						
	0	**0.25**	**0.5**	**0.75**	**1**	**1.5**	**2**
Cowpea	35.00±1.21 (70.0)	15.40±0.32 (30.8)	8.70±0.24 (17.5)	3.82±0.15 (7.64)	2.00±0.01 (4.0)	1.00±0.00 (2.0)	1.00±0.00 (2.0)
Bambara groundnut	32.00±1.51 (64.0)	15.73±0.56 (31.46)	9.25±0.35 (18.5)	3.96±0.11 (7.92)	2.25±0.03 (4.5)	1.00±0.00 (2.0)	1.00±0.00 (2.0)
Pigeon pea	25.00±1.26 (50.0)	15.56±1.3 (31.12)	9.75±1.22 (19.5)	4.81±1.18 (9.62)	2.75±0.81 (5.5)	1.50±0.69 (3.0)	1.00±0.00 (2.0)

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