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INTER AND INTRA-SPECIES SIMILARITY AND VARIATION IN *CALLOSOBRUCHUS* SPECIES EMERGENCE FROM MUNGBEANS, *VIGNA RADIATA* (L.) WILCZEK

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ABSTRACT
Mungbeans (*Vigna radiata* (L.) Wilczek) is a legume that belongs to the family, Fabaceae. The crop is commonly utilized in Asian continent and it has spread across the globe just like other important crops. This legume is prone to infestation by the genus *Callosobruchus.* This study investigated the inter- and intra-species similarity or variation in emergence potential of *Callosobruchus* species from mungbean. Three species of *Callosobruchus* (*C. maculatus*, *C. chinensis* and *C. rhodesianus*) and four strains of *C. maculatus* (Cameroon, Brazil, Yemen and Uganda strains) were used for the study. The test insects were allowed to oviposit separately on mungbeans and for each species/ strain, ten (10) seeds having hatched eggs were monitored individually. Total adult emergence per seed, day of commencement of emergence and cessation were monitored. Emergence Time of 50% of the total emerged population (ET_{50}) and the time for 50% seed loss (SLT_{50}) were also estimated. Generally, emergence cessation was on the 30th day; however, this was delayed in Yemen and Brazil strains of *C. maculatus*. The shortest SLT_{50} (3 Days) was observed in *C. chinensis* and the longest (7 days) in *C. maculatus* (Brazil and Yemen strains). *C. maculatus* (Uganda) had the shortest ET_{50} (2.81 days) and *C. maculatus* (Yemen) had the longest (6.35 days). *C. chinensis* had the highest adult emergence (26 adults) and *C. maculatus* (Uganda) had the lowest (9 adults). Based on SLT_{50} and ET_{50}, African populations of *Callosobruchus* species show potentials for early emergence from mungbeans. Therefore, the preservation of mungbean seeds from *Callosobruchus* species should be handled properly on the African continent.

Key words: Mungbean; *Callosobruchus*; species; strain; adult emergence; seed loss

INTRODUCTION

Mungbeans, *Vigna radiata* (L.) Wilczek, also known as green gram belongs to the family Fabaceae. It is a legume which originated from India in Asia (Feedipedia, 2016). Due to emigration, Asians have spread across the globe together with their foods which include mungbeans. This legume is highly susceptible to infestation by the genus *Callosobruchus.* Infestation by *Callosobruchus* species affects the visual and the nutritional values of grain legumes. The insect lays eggs which hatch into larvae which destroy the grains as a result of their biological activities. The emergent holes left behind after adults emergence make the grains unsightly. In addition, the feeding and the breeding activities of the insects aid mould growth and all of these may result in the reduction of the market value, germinability and even total obliteration of infested grains.

Callosobruchus species are major insect pests of several legumes. The genus consists of several cosmopolitan tropical and subtropical pests including *C. maculatus* (Fab.), *C. chinensis* (Linn.) and *C. rhodesianus* (Pic). *C. maculatus* is the most notorious and most prominent among this genus (Gbaye *et al* 2011). *C. chinensis* is also widely spread like *C. maculatus* but it is more prominent in Asia than the other species.

C. rhodesianus has more limited geographical distribution than the other two species with its infestation mainly in subtropical Southern Africa (Giga and Smith, 1983; Tuda *et al.*, 2005). However, there is evidence of its tendency to spread to tropical regions thereby enlarging its habitat spread due to its ability to tolerate tropical temperatures (Giga *et al.*, 1993; Gbaye *et al*., 2011).

Although all the aforementioned *Callosobruchus* species have the same life history, the environmental factors encountered such as temperature, humidity, type and size of host on which the species develop have affected them in various ways. These effects have been observed in their oviposition behaviour, fecundity, developmental period, progeny survival and adult weight (Howe and Currie, 1964; Chandrakantha and Mathavan, 1986; Giga and Smith, 1987; Timms, 1998; Soares *et al.*, 2007). However, all these parameters vary between species and their identified geographical strains (Dick and Credland, 1986; Messina, 1991; Mitchell, 1991; Giga *et al.*, 1993; Lobley-Taylor, 2000).

The life history of the insects begins with the laying of oval and flattened eggs which are glued to individual seeds. Though the total number of eggs laid by *C. chinensis* overlap with those of *C. maculatus* but *C. rhodesianus* lays fewer eggs than *C. maculatus* (Howe and Currie, 1964; Giga and Smith, 1983; 1991). The eggs hatch and the first instar larval bore through the chorion and seed coats into the seed. The larva is the major destructive stage and the entire larval to pupal period is lived in a single seed.

The situation of global food security is worsened in Sub-Saharan Africa and Asia through climate change which has been predicted to have the highest impact in these regions (Müller, 2009; Nelson *et al.*, 2010). These two continents have several developing nations whose teeming populace depend on legumes (to a large extent) for their protein needs. Hence in the face of global climate change, the challenge posed by *Callosobruchus* to legumes in these regions needs proactive measures. Cowpea is readily accepted as host by the three species when compared with other legumes such as mungbean (Giga *et al.*, 1993). However, there is an inherent suitability of mungbeans to *C. chinensis* (Jaenike, 1990). This study investigated inter-species and intra-species emergence potential of *Callosobruchus* species from mungbeans.

MATERIALS AND METHODS

Insect stock culture

This study was carried out in the Environmental Biology section of the School of Biological Sciences, University of Reading, United Kingdom. Three species of *Callosobruchus* and four strains of *C. maculatus* were investigated in this study (Table 1). The Cameroon strain of *C maculatus* was used as the reference strain..

Table 1. Species and strains of *Callosobruchus* used in the study

Species	Strain	Strain Continent of Origin
Callosobruchus maculatus	Brazil	South America
Callosobruchus maculatus	Cameroon	Africa (West)
Callosobruchus maculatus	Uganda	Africa (East)
Callosobruchus maculatus	Yemen	Asia (West)
Callosobruchus chinensis	Kenya	Africa (East)
Callosobruchus rhodesianus	Zimbabwe	Africa (South)

Hence it was used in the species comparison. The mungbeans used for culturing the insects was sterilized by freezing at -18°C for a period of 4 weeks. The mungbeans was later placed on the laboratory shelf to equilibrate for 2-3 days prior to use. Prior to the study, each species/ strain have been maintained on mungbeans for over 100 generations in a controlled temperature and humidity (CTH) insectary at 30±1°C and 55 ± 10% r.h.

Emergence experiment

The test insects (80 adults) were allowed to oviposit on 150 grammes of mungbeans in the culture bottles (500 ml glass jars). The adults were later sieved out and the culture left for one week to allow the hatching of the eggs. Hatched *Callosobruchus* eggs were identified by their whitish chorion instead of the initial colourless/ transparent appearance. From the culture bottle, ten (10) mungbean seeds having more than two hatched eggs were selected for the experiment. This was done separately for each species/ strain with each seed serving as a replicate (i.e. 10 replicates). The infested seeds were placed individually in different 8.5ml sterilin plastic vials with caps. These vials were placed in the insectary and monitored for emergence. The daily emergence from each seed was recorded for 10 days commencing from the 23rd day post culture set up to the 32nd day.

The following parameters were recorded for each species or strain, they are: adult emergence per seed; total number of seeds without emergence; day of emergence commencement and cessation.

Assessment of seed loss time (SLT)

The time taken for the insects to cause 50% seed loss (SLT_{50}) was assessed by observing the number of days it took adult beetle to emerge from the 1st five mungbean seeds out of ten. This was used to estimate how fast it took 50% of the seed to be riddled with emergent holes. This was taken as the rate of mungbean seed loss to each population (species/strain) of *Callosobruchus*.

Statistical Analysis

The average number of emergence per seed for each species or strain was calculated. Analysis of Variance was performed on the daily emergence data, all the species and strains were analysed together. Minitab v17 was used to analyse the data and also used to plot the distribution curve of the number of emerged adults per mungbean seed.

Emergence Time of 50% Adult (ET_{50})

Line graph of daily adult emergence was plotted using Microsoft Excel and the regression for each population was determined. To know how fast it would take for generational turn over to occur, the regression equations were used to estimate ET_{50} (Emergence Time of 50% of the total emerged adult) for each population.

RESULTS AND DISCUSSION

The trend of emergence of *Callosobruchus* species from mungbeans is presented in Figure 1. Generally, adult emergence commenced on the 24th day after oviposition; however it was a day earlier (i.e. 23rd day) for both the Brazil and the Uganda strains of *C. maculatus*. All species of *Callosobruchus* reached their maximum emergence on the 8th day of monitoring (30th day from oviposition) (Figure 1a). However, for *C. maculatus* strains two groups were observed. Uganda and Cameroon strains (i.e. African strains) reached their maximum emergence earlier on the 6th and 8th day respectively and these were the 28th and 30th day respectively after oviposition (Figure 1b). In contrast, Brazil and Yemen strains' emergence continued till the last day of monitoring (10th day) (Figure 1b). This observation on *C. maculatus* strains is in line with the findings of Messina *et al* (2018) who had earlier observed that African populations of this species have tendency for the acceptance of less preferred hosts.

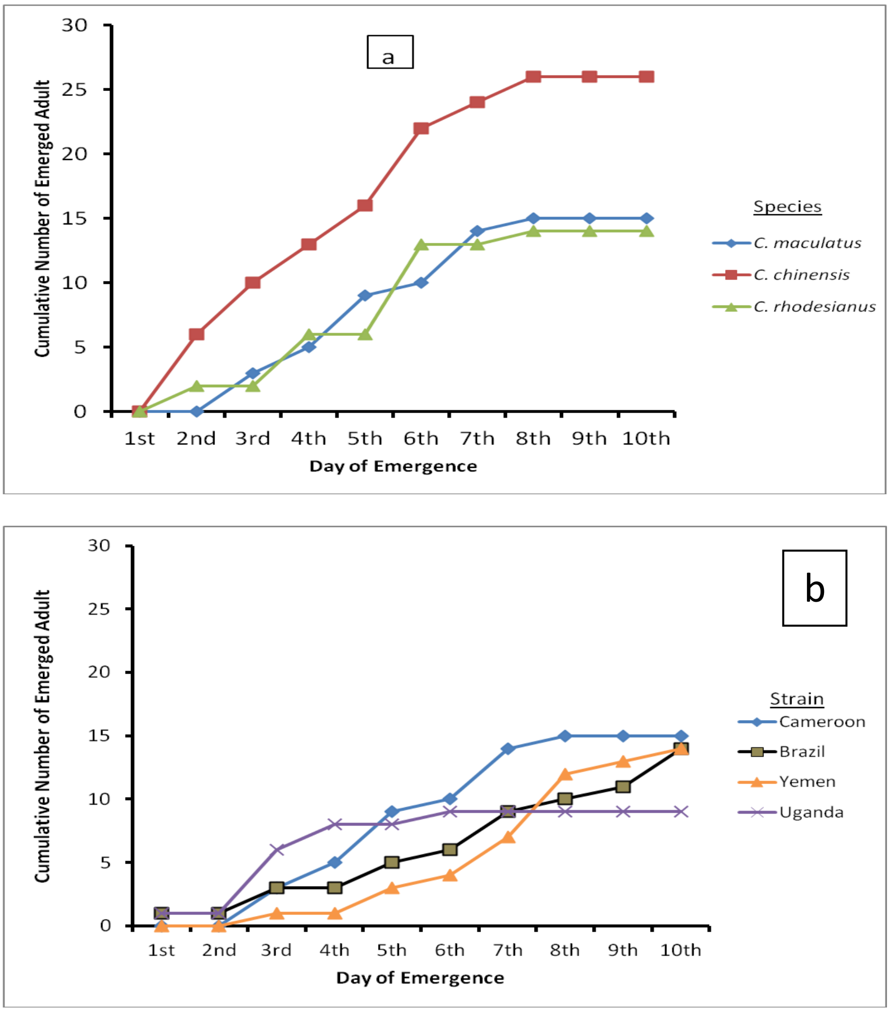

Figure.1: Adult emergence trend of (a) *Callosobruchus* species and (b) *C. maculatus* strains from mungbean seeds

Mungbean is an exotic legume to Africa unlike cowpea which is mostly preferred by *C. maculatus.*

If the emergence monitoring was beyond 10 days, per adventure, both Brazil and Yemen strains might have continued to emerge. This is yet to be determined. This means if a control strategy is targeted at emerged adults for eight days, there is tendency that some individuals of these two strains would evade the control strategy and start fresh infestation. There were significant differences ($p < 0.05$) in the emergence rate of *C. chinensis* and two strains of *C. maculatus* (Yemen: from Day 4 to Day 7; Uganda: from Day 7 to Day 10).

The highest number of adult insect emergence was obtained in *C. chinensis* (26 insects) while the least (i.e. 9 adults) was obtained in *C. maculatus* (Uganda) (Table 2). Figure 2 also shows that mungbean is highly susceptible to *C. chinensis* and it supports its proliferation. This is because from 70% of the monitored seeds, 3 or more adults emerged from a single mungbean. This same rate was also observed in 40% of the seeds monitored for *C. rhodesianus* emergence. However, only 10% of seeds for *C. maculatus* (all strains) had 3 adults emerged from a single mungbean seed (Figure 2). It has been argued that mungbean is an unsuitable host for *C. maculatus* (Guedes *et al*., 2003). Figure 2 also shows that the distribution of *C. chinensis* adult emergence was negatively skewed (-1.66). This means that most of the seeds have more *C. chinensis* emergence than the observed mean (2.6 adult/seed). This is not surprising because mungbeans and *C. chinensis* tend to have their origins from Asia (Gbaye *et al*, 2011; Feedipedia, 2016; CABI, 2018). *C. chinensis* is a serious pest of mungbeans in Asia. They might have evolved together over time, thus giving this species an inherent ability to utilize mungbeans (Khattak *et al*, 1987; Jaenike 1990; Gbaye *et al*., 2011). Secondly, *C. chinensis* was observed to be smaller in size than the other species/ strains; given the small size of mungbean seed, it is not out of place to have more adult *C. chinensis* emerging from a single seed than the other species.

Table 2: Total adult emergence of *Callosobruchus* from mungbeans and seeds without adult emergence

Species/ Strain	Total Number of Emerged Adult	Seed without Adult Emergence (%)
C. maculatus Cameroon	15	10
C. maculatus Brazil	14	20
C. maculatus Yemen	14	10
C. maculatus Uganda	9	40
C. chinensis	26	10
C. rhodesianus	14	50

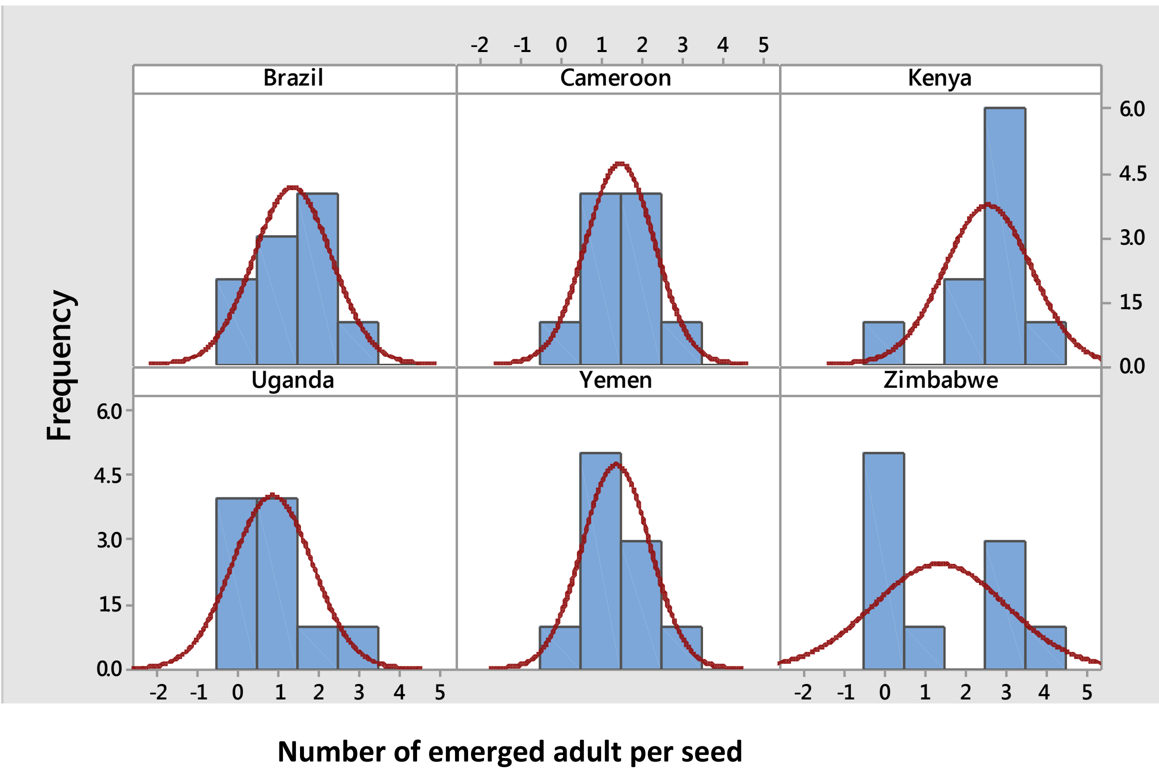

Figure 2. Histogram with normal curve showing distribution of number of emerged adult *Callosobruchus species* and *C. maculatus* strain per mungbean seed after 10 days (N= 10)

Note: Kenya = *C. chinensis* (Kenya strain); Zimbabwe = *C. rhodesianus* (Zimbabwe strain)
Brazil, Cameroon, Uganda and Yemen = *C. maculatus* strains

It was observed that *C. chinensis* and *C. rhodesianus* have steep increase in cumulative adult emergence from the 5th to the 6th day (between 27th and 28th day after oviposition) which was not observed in any strain of *C. maculatus* (Figure 1). This period could be targeted for the management of these two species to keep them below economic threshold level.

Table 2 shows that the total adult emergence of *C. rhodesianus* (14 adults) is about the same number for all *C. maculatus* strains tested (except Uganda), however, 50% of the monitored seeds with *C. rhodesianus* eggs had no adult emergence. Giga and Smith (1991) observed a continuous decline in *C. rhodesianus* emergence per cowpea seed at a high larvae density. Increased larvae density is known to affect adult emergence negatively (Oliveira *et al*, 2015).

Since mungbean is a smaller seed than cowpea, larvae density might have caused the high rate of non-emergence observed in *C. rhodesianus* in this study. On the other hand, *C. maculatus* (Uganda) with a similar high percentage of seeds without emergence (40%) had only 9 emerged adults. This shows a very low suitability of mungbeans for the Uganda strain of *C. maculatus* (Giga *et al.*, 1993) when compared to the other stains. Observations on the other strains in this study shows 10-20% non-emergence which is in line with Panzarino *et al* (2011) observation of *C. maculatus* emergence from chickpea.

The time taken for 50% seed loss (SLT_{50}) is presented in Figure 3. The shortest SLT_{50} was observed in *C. chinensis* (3 days) and the longest in *C. rhodesianus* (6 days). The African strains (Cameroon and Uganda) of *C. maculatus*, recorded shorter SLT_{50} of 4 days each while their counterparts from Brazil and Yemen recorded 7 days each. This is a further proof of *C. chinensis* superior pest status than other *Callosobruchus* species on mungbeans. Hence buttressing its ability to utilize and invariably destroy mungbean seed (Khatttak *et al*, 1987) faster than other species. The rate of mungbean seed loss to the African populations of *C. maculatus* was almost half of the time required by the other strains. It is therefore exigent for mungbean farmers and merchants on the Africa continent to take every precaution to prevent the infestation of the crop by *C. maculatus*. The result of the emergent time of 50% of the total adult beetle is presented in Table 3. The result shows that the Uganda strain of *C. maculatus* had shorter ET_{50} (2.81 days) than *C. chinensis* (4.19 days). Apparently, this was due to the small number of total adult emergence recorded for this strain (9 adults, $R^2 = 0.6900$ {Table 3}) when compared with that of *C. chinensis* (26 adults, $R^2 = 0.9289$ {Table 3}).

Table 3. Emergence time of 50% (ET_{50}) of adult *Callosobruchus* species and *C. maculatus* strains from mungbean seeds

Species	Strain	Regression Equation	R^2	ET_{50} (Days)
C. maculatus	Brazil	y = 1.4606x - 1.7333	0.9665	5.98
	Cameroon	y = 1.9879x - 2.3333	0.9411	4.95
	Yemen	y = 1.7636x - 4.2	0.9084	6.35
	Uganda	y = 0.8909x + 2	0.6900	2.81
C. chinensis	Kenya	y = 2.9879x + 0.4667	0.9289	4.19
C. rhodesianus	Zimbabwe	y = 1.8061x - 1.5333	0.8958	4.73

On the overall, *C. maculatus* had longer ET_{50} on mungbeans than the other species. As earlier observed, the size of *C. maculatus* is bigger than those of *C. chinensis*, hence, its larvae will need more food, space and time for development than *C. chinensis.* Hence, *C. chinensis* will have a faster generational turn over than the other species. This will invariably aid its population growth on mungbeans which is a smaller seed compared to cowpea. The type and size of host on which *Callosobruchus* species develop affect their developmental periods, progeny survival and adult weights (Chandrakantha and Mathavan, 1986; Giga and Smith, 1987; Timms, 1998; Soares *et al.*, 2007). These parameters also vary between species and their geographical strains (Messina, 1991; Mitchell, 1991; Lobley-Taylor, 2000). Again, the African strains of *C. maculatus* had lower ET_{50} than the other two strains. For instance, the ET_{50} for the Cameroon strain is about 24 hours and 34 hours less than those of Brazil and Yemen strains respectively. These hours are enough for *C. maculatus* to lay enough eggs which can lead to massive seed loss due to population explosion.

CONCLUSION

This study revealed inter-species and intra-species emergence similarity and variations of *Callosobruchus* species from mungbeans. On this legume, *C. chinensis* remains the most pestiferous of the three species investigated and the African populations of *C. maculatus* have a high potential of causing quick havoc to this legume. Presently, mungbean is cultivated in so many–countries in Africa and *C. chinensis* has also been reported in some African nations (Feedipedia 2016; CABI 2018). Therefore, the preservation of mungbean seeds from *Callosobruchus* species should be handled properly on the African continent.

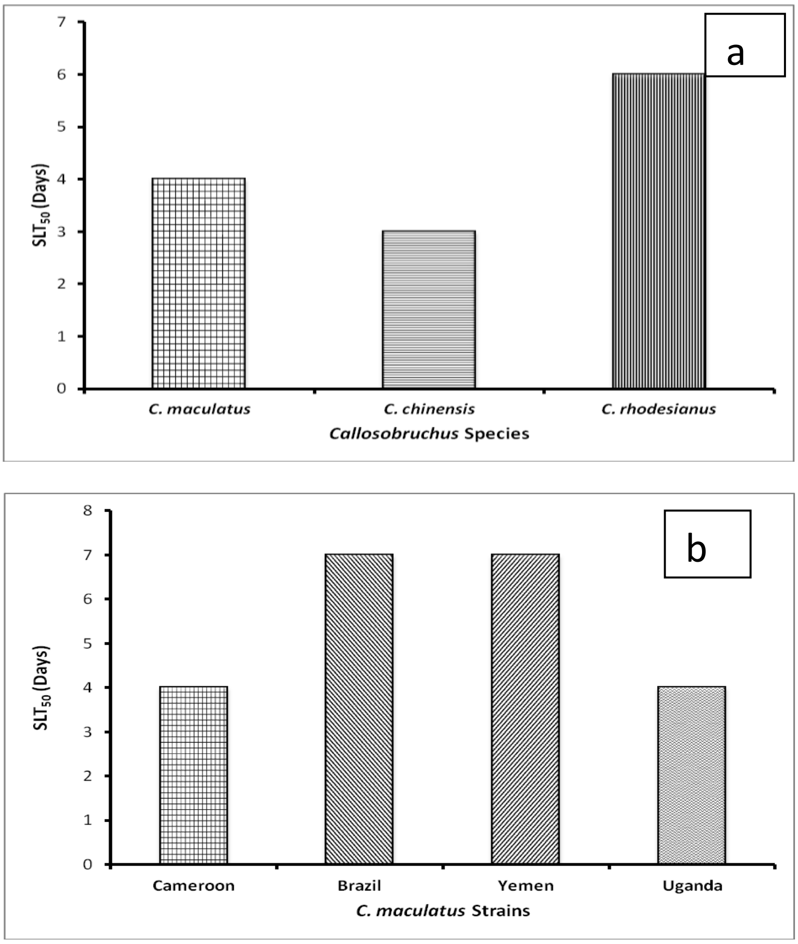

Figure 3: Fifty percent Seed Loss Time (SLT_{50}) of mungbean seeds caused by (a) *Callosobruchus* species and (b) *C. maculatus* strains

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