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Potential of five wild legume species as alternative rearing substrates of *Uscana lariophaga* Stef. (Hymenoptera: Trichogrammatidae), a promising biocontrol agent of Bruchids pest of cowpea in West Africa

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Abstract
Callosobruchus maculatus (Coleoptera: Chrysomelidae, Bruchinae) is the major pest of stored cowpeas in West Africa. Biological control using its egg parasitoid *Uscana lariophaga* Stef. (Hymenoptera: Trichogrammatidae) is currently under investigation but faces limitations due mainly to the difficulty of mass production. Research was conducted under laboratory conditions to evaluate the suitability of five wild legume seeds for *Callosobruchus maculatus* Fab. and its egg parasitoid rearing in comparison to two varieties of *Vigna unguiculata*, (*KXV 61-1* and *Local Moussa*) used as control host plants. The seeds of the wild legumes physically described using texture, weight, size and color were different from cowpea seeds. The analysis of *C. maculatus* oviposition in no-choice experiments showed that eggs were laid on all the legume seeds tested. However, the wrinkled cowpea seeds were more infested (77.50±2.89 eggs) than the wild legumes smooth seeds, the lowest mean number of eggs (9.15±0.98) being observed on *A. gourmaensis* flat seeds. Regarding the reproductive potential of *U. lariophaga*, the eggs of *C. maculatus* laid on all the five wild legumes may be parasitized and allow the development of the progeny of the parasitoid. Based on the intrinsic rates of natural increase, three wild legume species (*A. nilotica*, *A. gourmaensis* and *A. macrostachya*) allowed a development of *U. lariophaga* equivalent to that obtained with the seeds of the cowpea varieties used. Our results provide a basis for the development of a method of mass production of the egg parasitoid *U. lariophaga* using the most promising wild legumes on which future research is expected to intensify.

Keywords: *Vigna unguiculata*, *C. maculatus*, wild legumes, *U. lariophaga*, alternatives diets, mass production

1. Introduction

Bruchids (Coleoptera: Bruchidae) are common storage pests of various grain legumes throughout the world [1-4]. Among them, *Callosobruchus maculatus* F. (Coleptera: Bruchidae) is reported to be the most damaging pest of stored legume seeds, especially cowpea, *V. unguiculata* (L.) Walp. in the tropics and subtropics [5-7]. In West Africa, *Callosobruchus maculatus* (F.) mainly develops on cowpea seeds and is considered as the most serious pest of this crop in storage [8-11]. Host plants are primarily cowpea while soya beans, Bambara groundnuts and other legumes are secondary hosts [6, 12, 13]. Infestation usually occurs in the fields [8, 14, 15] and continues during post-harvest storage were several generations overlap and damage stored cowpeas [10].

Biological control of this bruchid pest using the oophagous parasitoid *U. lariophaga* is currently under investigation. Recent studies have shown that *C. maculatus* populations can be controlled in cowpea crops and in experimental seesd storage systems using augmentative releases of *U. lariophaga* [16]. However, the effective application of biological control on a large scale requires the availability of biological control agents in quantity and quality. This is only possible if an economical and efficient mass rearing method is developed. Until now, parasitoids were produced only using cowpea as a development substrate for bruchid egg laying [16]. However, it does not seem economically acceptable to produce parasitoids using large quantities of cowpea [17]. Hence, the idea of taking an interest in wild legumes, generally little exploited and of less economic value than cowpeas, provided that they are suitable for

C. maculatus egg laying and the development of *U. lariophaga*. One could then wonder if the five wild legume species targeted by our study could not effectively replace cowpea for rearing the parasitoid *U. lariophaga*. Previous studies have already shown that the oviposition, growth and development of *C. maculatus* vary depending to the hosts [18, 19]. It has also been shown that *U. lariophaga* can develop on beetle eggs deposited on secondary hosts such as chickpea and pigeonpea [20]. An ideal legume species for *U. lariophaga* production should, first be accepted by females of *C. maculatus* for egg laying. Then, the parasitoid females should also accept these eggs for parasitism and finally it would be necessary that the parasitoid be able to develop successfully on/inside these eggs.

The present study aims to assess the potential of five local wild legume species for the egg laying of females *C. maculatus*, which would allow *U lariophaga* to develop. The fecundity of *C. maculatus* females was thus compared on the seeds of two cowpea varieties and those of five wild legume species from Burkina Faso. Rearing trials of parasitoids were also carried out when the alternative host plants were accepted and infested with *C. maculatus* eggs. The results should make it possible to identify the best wild legumes candidate for developing alternative substrates for mass production of parasitoids.

2. Material and methods

2.1 Origin and physical characteristics of the seeds of wild legume species and cowpea varieties used

The seeds of wild legumes used: *Acacia nilotica* (L.) Willd., *Acacia sieberiana* DC, *Acacia erythrocalyx* Brenan, *Acacia gourmaensis* (A.) Chev. and *Acacia macrostachya* Reichenb were purchased from the National Center for Forest Seeds in Ouagadougou (Burkina Faso), based on their availability, but also on their inedibility for human consumption. The cowpea (*Vigna unguiculata* L. Walp.) seeds of the varieties *KVX 61.1* and *Moussa Local* were obtained from the agricultural research center of Kamboinsé. All seeds were then carefully sorted to remove those that carry eggs or beetle larvae. The healthy remaining seeds were placed in a freezer at a temperature of -18 °C for two weeks to eliminate any initial infestation.

Prior to the experiments, the seeds of these legumes were described and compared based on texture, color, weight, diameter, shape and thickness. The texture was qualitatively determined by visual observation and touch by rubbing the seeds within the fingers. For weight determination, 150 seeds of each legume were weighed individually using an OHAUS GT 4100 D electronic scale. The diameter and thickness were measured on a sample of 500 seeds individually measured using a caliper from SKOLE. Visual observations made it possible to determine the shape and color variations between legume seeds.

2.2 Experimental conditions, insects' origin and rearing

All the experiments were carried out in the Laboratory of Fundamental and Applied Entomology in an incubator *LMS* at the University Joseph KI-ZERBO in Burkina Faso. The strains of *C. maculatus* and *U. lariophaga* used in this study were obtained from cowpea, variety *KVX 61-1*, collected in the experimental fields from Gampela near Ouagadougou in 2011 and maintained in laboratory by continuous rearing. Individuals of *C. maculatus* were reared in Plexiglas boxes (18×11 × 4 cm) containing healthy cowpea Moussa local seeds on which the females could oviposit. Cultures were then maintained in an incubator *LMS* with a temperature of 32±2 °C and a relative humidity (Rh) of 27%. Cowpea seeds carrying 2-day-old *C. maculatus* eggs were isolated and placed in presence of *U. lariophaga* to be parasitized. The parasitized eggs were monitored under the same rearing conditions and the successive generations used for the experiments or for maintaining the strain.

2.3 Egg-laying capacity of *C. maculatus* females on seeds of wild legumes

Ten (10) healthy seeds of each legume species and both cowpea varieties were placed in experimental glass tubes of 30 cm^3. To test oviposition ability into no-choice experiments, one (01) couple of newly emerged *C. maculatus* was introduced into each glass tube for 24 hours. The seeds were renewed at the end of each 24 hours-period until the death of the female. The removed seeds were isolated and the number of *C. maculatus* eggs counted after 7 days required for the eggs to hatch in our experimental conditions. The hatching rate was also determined. To compare the egg-laying and hatching ability of *C. maculatus* on wild legumes compared to cowpea, an acceptability index was calculated using the formula [13]:

AI = 1- | (Pni-Ppt) | / Pni; where *Pni* is the value of the number of eggs laid on cowpea and *Ppt* the number of eggs laid on the wild legume tested. When this index is close to 1, the wild legume species offers the same possibilities as cowpea for *C. maculatus*; and when it is close to zero, it indicates that *C. maculatus* is having trouble accepting this legume.

2.4 Reproductive potential of *U. lariophaga* on *C. maculatus* eggs laid on wild legume seeds

One (01) pair of newly emerged and non-nourished *U. lariophaga* was introduced into a glass tube containing ten (10) seeds of cowpea or of each of the 5 wild legumes carrying twenty (20) fresh eggs (12 hours old)) of *C. maculatus* that female parasitoids could parasitize. *C. maculatus* eggs were daily replaced until the death of the female parasitoid. The number of *C. maculatus* eggs that turned black or brown was used to determine the parasitism due to *U. lariophaga*. From the number of parasitoids emerging from black/brown eggs, mortality could be estimated. In each case, the development time of the parasitoid was determined by counting the total number of emerged parasitoids at the end of the experiment considering their respective day of emergence.

The intrinsic rate of natural increase (r_m) was then determined using the following formula [21]: r_m=lnx/(t+1/2p), where *ln* is the Naperian logarithm, *x*=number of females' eggs surviving to adulthood, *t*=development time (days) and ***p***=oviposition period (days). The sex ratio was also determined.

2.5 Data analysis

The data collected was subjected to an analysis of variance (ANOVA) using SAS software version 9.1. Significantly different means were separated using the Fischer LSD test at the 5% level.

3. Results

3.1 The different legume seeds characteristics

The morphological characteristics of the seeds of legume species and/or varieties used are compared in table 1. There were significant differences in mean diameter *(F=3.4; p=0.0024)*, weight *(p<0.0001)* and thickness *(F=68.77; p<0.0001)* of the different legume seeds. The seeds of *Acacia macrostachya*, *A. gourmaensis* and *A. erythrocalyx* had a significantly larger diameter than that of other legumes. Both cowpea variety seeds were thicker than those of wild legumes. They also weighed more than wild legumes, except the seeds of *A. sieberiana*, which had a weight similar to that of the seeds of the local Moussa cowpea variety.

Regarding the qualitative parameters, all wild legumes had smooth seeds while those of both cowpea varieties were wrinkled. Wild legumes tended to have flat or oval seeds, but cowpea seeds were kidney shaped for both varieties. The color seemed very variable among the seeds of all the legumes tested but cowpea seeds tended to be white while wild legume seeds were rather dark.

Table 1: Morphological characteristics of different host plants seeds used as hosts for *C. maculatus* and *U. lariophaga* rearing.

Legume species	(Diameter) (mm)	Thickness (mm)	Weight (g)	Texture	Shape	Colour
V. unguiculata Moussa Local	6.08 ± 0.02^{bc}	4.64 ± 0.02^{a}	0.18 ± 0.002^{a}	wrinkled	kidney-shaped	White
V. unguiculata var. KVX 61-1	6.02 ± 0.02^{bc}	4.40 ± 0.02^{ab}	0.16 ± 0.002^{b}	wrinkled	kidney-shaped	White with brown hilum
Acacia nilotica	6.23 ± 0.02^{bc}	3.69 ± 0.02^{c}	0.15 ± 0.002^{c}	smooth	shapeless	black
Acacia sieberiana	5.75 ± 0.04^{c}	4.10 ± 0.02^{b}	0.18 ± 0.002^{a}	smooth	oval	Dark gray
Acacia erythrocalyx	6.68 ± 0.05^{abc}	2.38 ± 0.02^{d}	0.13 ± 0.002^{d}	smooth	Flat	Dark brown
Acacia gourmaensis	8.17 ± 1.39^{a}	2.35 ± 0.36^{d}	0.08 ± 0.001^{f}	smooth	Flat	Brown
Acacia macrostachya	7.91 ± 0.03^{ab}	1.74 ± 0.01^{e}	0.10 ± 0.005^{e}	smooth	Flat	Brown
PLSD (5%)	0.0024	<0.0001	<0.0001			

Means (± SE) within columns followed by different letters significantly differ according to the PLSD Fischer test at the 5% level.

3.2. Egg-laying capacity of *C. maculatus* females on seeds of wild legumes

Females of *C. maculatus* laid eggs on all the legume seeds tested (Fig. 1). However, the mean number of eggs was significantly lower on the wild legume seeds, which also significantly differed from each other (Fig. 1). The seeds of wild legumes were ranked in decreasing order of the number of *C. maculatus* eggs received as follows: *A. nilotica*, *A. sieberiana*, *A. erythrocalyx*, *A. macrostachya* and *A. gourmaensis*, respectively (Fig. 1).

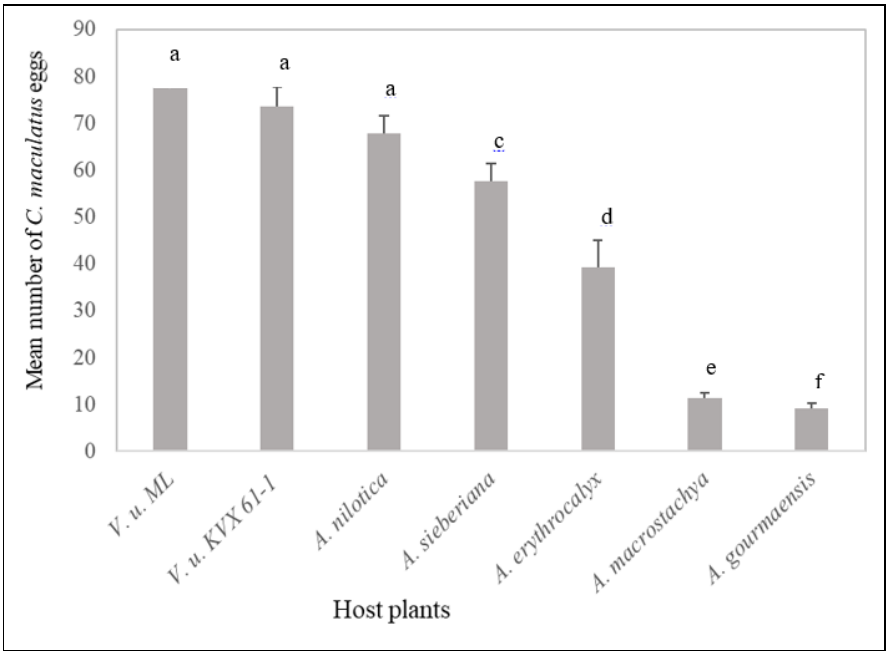

Fig 1: Comparison of the mean number of eggs (±SE) laid by *C. maculatus* females on different host plant seeds. *V. u. ML*: cowpea Moussa Locale variety; *V. u. KVX61-1*: cowpea KVX61-1 variety.

The acceptability index applied to the number of eggs laid by *C. maculatus* females is <1 for all the wild legumes used (Table 2). However, the seeds of *A. sieberiana* and *A. nilotica* with index of 0.69 and 0.78 respectively, offered the best potential for *C. maculatus* eggs compared to the other three legume species where AI varies from 0.12 to 0.48 (Table 2).

Table 2: Acceptability index applied to *mean number of C. maculatus* eggs

	A. sieberiana	*A. nilotica*	*A erythrocalyx*	*A. gourmaensis*	*A. macrostachya*
AI	**0,69**	**0,78**	0,48	0,12	0,15

AI= Acceptability index

The percentage of eggs hatching was relatively high (>60%) regardless of the legume seeds considered (Fig.2). The higher values (75%) were obtained on cowpea *KXV 61-1* variety, *A. erythrocalyx* and *A. macrostachya* seeds *(F=3.81; p=0.0008*). The seeds of *A. gourmaensis* received a significantly lower number of *C. maculatus* eggs (Fig 2).

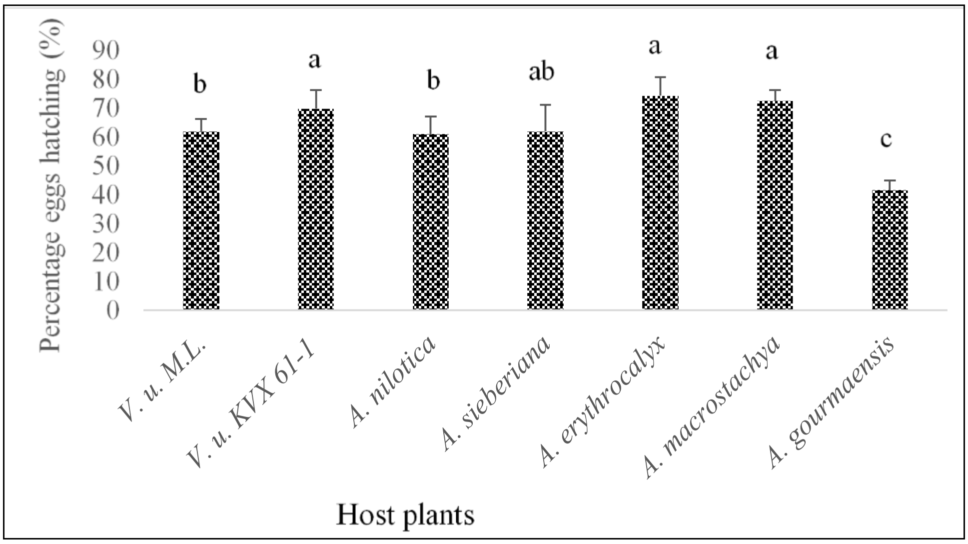

Fig 2: Variation of mean percentage (±SE) of *C. maculatus* eggs hatching on different host plants seeds. *V. u. ML*: cowpea Moussa Locale variety; *V. u. KVX61-1*: cowpea KVX61-1 variety.

3.3 Reproductive potential of *U. lariophaga* on *C. maculatus* eggs laid on wild legume seeds *Parasitism on C. maculatus eggs*

U. lariophaga females parasitized *C. maculatus eggs* deposited on all legume seeds in different proportions. The highest level of parasitism was recorded on *C. maculatus* eggs developing on *A. gourmaensis* and *A. macrostachya* seeds (Table 3). When *C. maculatus* eggs were laid on the seeds of all other legume species including both cowpea varieties, they were less parasitized by *U. lariophaga* (Table 3).

3.4 Development time

Development was successful on all alternate substrates. The *U. lariophaga* developmental time was <6 days in all the cases and did not significantly varied among the legume seeds used (Table 3). Therefore, the development time was similar for *U lariophaga* developing on hosts located on both cowpea varieties and wild legume seeds as well (Table 3).

3.5 Lifespan

The parasitoids emerging from the parasitized eggs on the seeds of all the legumes used had a relatively short lifespan <3 days. However, there was no significant difference between the lifespans obtained regardless of the host legume species, including both cowpea varieties (Table 3).

3.6 Estimated pre-imaginal mortality

This mortality was significantly higher for *U. lariophaga* developing on *C. maculatus* eggs laid on the seeds of both cowpea varieties (Table 3). Lower mortality rates were noticed when the host legume plants used were *A. gourmaensis* and *A. macrostachya*.

3.7 Sex ratio

There was no significant difference for this parameter among parasitoids emerging from all the experimental settings. The sex ratio was close to 0.5 (Table 3).

3.8 Intrinsic rate of natural increase (R_m)

Interestingly, the r_m of *U. lariophaga* was similar on the eggs of *C. maculatus* laid on the seeds of three wild legumes, *A. nilotica*, *A. gourmaensis* and *A. macrostachya* and those of the two cowpea varieties used (Table 3). *A. sieberiana* and *A. erythrocalyx* yielded the lowest r_m values.

Table 3: Life history parameters of *U. lariopaha* parasitizing *C. maculatus* eggs reared on cowpea and wild legume seeds. N=20 replications.

	***V. unguiculata* var. Moussa Local**	***V. unguiculata* var. KVX 61-1**	***A. nilotica***	***A. sieberiana***	***A. erythrocalyx***	***A. gourmaensis***	***A. macrostachya***	***P & f Values***
Mean number of parasitized eggs	23.7±1.51^a	25.25±1.87^a	16.35±1.59^b	17.55±1.89^b	18.20±1.57^b	7.3±0.55^c	8.9±0.83^c	*p<0.0001(s) ; f=16.59*
Mean rate of parasitism (%)	41.88±3.76^b	41.32±2.26^b	40.27±4.63^b	32.73±2.79^b	42±4.40^b	83.39±4.27^a	72.49±5.4^a	*p<0.0001 (s); f=18.92*
Development time	4.95±0.25^a	5.35±0.17^a	5.55±0.11^a	5.45±0.15^a	5.5±0.2^a	5.5±0.2^a	5.2±0.17^a	*p=0.31 (ns); f=1.19*
Lifespan	2.55±0.17^a	2.45±0.18^a	2.3±0.18^a	2.60±0.15^a	2.5±0.18^a	2.55±0.2^a	2.2±0.17^a	*p=0.75 (ns); f=0.60*
Pre-imaginal mortality	8.45±1.13^{ab}	9.35±1.38^a	3.65±0.62^{cd}	5.5±0.90^{bcd}	4.2±1.03^{cd}	1.9±0.40^d	2.15±0.45^d	*p<0.0001 (s); (s) f=7.99*
Sex ratio (*Sr*)	0.53±0.02^a	0.54±0.03^a	0.52±0.06^a	0.55±0.05^a	0.46±0.05^a	0.47±0.08^a	0.53±0.07^a	*p=0.92 (ns) ; f=0.36*
Intrinsic rate of natural increase (r_m)	0.256±0.004^a	0.257±0.004^a	0.259±0.003^a	0.253±0.003^a	0.253±0.004^a	0.253±0.005^a	0.264±0.004^a	*p=0.48 (ns); f=0.93*

Means (±SE) within rows followed by different letters significantly differ according to the PLSD Fischer test at the 5% level.

4. Discussion

Our results showed that wild legumes could be used as alternative hosts for *C. maculatus* oviposition and for the egg parasitoid *U. lariophaga* development. Indeed, *C. maculatus* females were able to lay on the seeds of all the legumes tested, with variable acceptability levels. Egg laying behavior of insect females on unusual hosts is a key factor that may allow insects to successfully vary their host spectrum, provided their larvae be able to develop on these hosts [24, 25]. The genus *Callosobruchus* occurs on a wide range of host plants in general [26], which also reflects its high adaptability [12].

The analysis of the oviposition behavior of *C. maculatus* revealed that females laid significantly more eggs on *A. nilotica*, *A. sieberiana* and *A. erythrocalyx* seeds compared to those of *A. macrostachya* and *A. gourmaensis*. Except for *A. nilotica* seeds, the number of eggs was lower on wild legume seeds in comparison to cowpea, the usual host. This observed preference of *C. maculatus* females could be explained by the difference in seeds' physical characteristics [27-29] which were found to be quite different. The size of host seeds is known to influence the number of eggs laid by *C. maculatus*, the larger seeds receiving generally more eggs [30, 31]. Likewise, a study comparing the egg laying behavior of *C. maculatus* on cowpea seeds and those of some unusual host plants (voandzou and pigeon pea), showed that females lay more eggs on *Vigna subterranea* seeds (larger size) than *Cajanus cajan* and cowpea seeds [12]. However, the seeds of several wild legumes, although larger than cowpea seeds, were less infested, which indicates that factors other than size also come into play. Female fecundity may also be influenced by the shape, color and texture of the seed coat [29, 32] and *C. maculatus* females may have variable responses for a particular host substrate [33]. On the other hand, *C. maculatus* females lay fewer eggs on the flat seeds because of the inaccessibility of the two faces or the small size of the available surface. Smooth seeds also carry fewer eggs because of the difficulty of adhesion to the seed coat [31]. Chemical characteristics [34, 26] and the nutritional quality of the seed coat [35] may also influence egg laying.

Regarding the ability of *U. lariophaga* to parasitize and develop in the eggs of *C. maculatus* deposited on the seeds of the five species of wild legumes tested, it appears that these eggs may well be parasitized and allow the development of the progeny of the parasitoid. Parasitism rates higher than those obtained on cowpea seeds were even observed with *A. gourmaensis* and *A. macrostachya*. Unfortunately, these two legume species were also the less infested by *C. maculatus* eggs. Based on an analysis of intrinsic rates of natural increase, a synthetic parameter determining the ability of the insect to multiply, it appeared that three wild legumes species (*A. nilotica*, *A. gourmaensis* and *A. macrostachya*) allowed a development of *U. lariophaga* equivalent to that obtained with the seeds of the cowpea varieties used. These results are original because the available data on the biodemographic parameters of *U. lariophaga* [22, 23] were obtained using cowpea as a host plant for Bruchids. Our results provide a basis for the development of a method of mass production of the egg parasitoid *U. lariophaga* using the most promising wild legumes. In this regard, it is interesting to note that *A. nilotica* was the most accepted legume for egg laying of *C. maculatus* and is also one of the legumes allowing the best intrinsic rate of natural increase. The development of a mass production method of parasitoids is however complex and requires more precise studies. Previous studies have identified the main challenges that must be faced in developing an effective method of mass rearing of parasitoids. These are the high cost of production, the lack of effective techniques for producing natural enemies using an artificial diet, the difficulty in maintaining the effectiveness of the parasitoids produced even after several generations, superparasitism which can reduce parasitism efficacy, change in behavior of parasitoids developed on alternative hosts, poor nutritional quality of an artificial diet and its potential contamination by pathogens [36-38]. Our results, although encouraging, should lead to additional studies aimed above all at evaluating the viability, behavior and performance of parasitoids produced from targeted wild legumes.

5. Conclusion

The results obtained from this study clearly show that the females of *C. maculatus* accept the hosts available for oviposition. These results indicate that *U. lariophaga* may be reared on *C. maculatus* eggs laid on the seeds of all wild legumes tested. Once *U. lariophaga* parasitizes eggs laid on the seeds of most of these legumes, it develops in the same way as on cowpea seeds and produces viable offspring. These results open interesting prospects for the mass production of *U. lariophaga* to support a possible program of fields' release of this promising biocontrol agent. The potential legumes on which research is expected to intensify are *A. nilotica*, *A. gourmaensis* and *A. macrostachya*.

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