The typing and the mitogenic potentials of *Lens cinarus* and *Phaselius aureus* seed lectins

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Abstract

Legium Lectins are widely used as a model system for protein- protein, protein-carbohydrate interactions. So two legium seeds were elected, as *Lens cinarius* and *Phaselius aureus* Roux for detection of their lectins .Such lectins were extracted, separated and partially characterized as LCL and PAL. respectively. Two prokaryotic cellular systems *Klebsiella pneumoniae* and *Escherichia coli* and two eukaryotic cellular systems the human erythrocytes as well as the avian T-lymphocyte, were elected.

Cell typability were studied on *K.pneumoniae, E.coli* and human erythrocytes , while T-cell mitogenicity were checked by lectin skin test in avian wing system. Two typing patterns were observed in both *K. pneumoniae* and *E.coli*. togther with LCL untypable isolates of *K. pneumoniae* and *E.coli* . Pantypability was identified in human erythrocyte in both of LCL and PAL. Positive mitogenic responses were noted in the avian wing test. Thus, both LCL and PAL have typing and mitogenic potentials. Which could be due protein- protein or protein-carbohydrate interactions.

الخلاصة

تستخدم لاكتينات البقول بوصفها نظام نموذج تفاعل البروتين- بروتين وتفاعل بروتين كاربوهيدرات وبناءا عليه جرى اختيار نوعين من بذور البقول وهما بذور العدس *L.cinarius* و الماش *P.aureus* للتحري عن وجود اللكتينات فيها . استخلصت وفصلت ووصفت جزئيا هذه اللكتينات وتم وسمها بLCL وPAL . وجرى اختبار نظامين خلويين من أولية النواة *K.pneumoniae* و*E.coli* للكشف عن القابلية التنميطية ونظامين من حقيقية النواة احدهما للكشف عن القدرة التنميطية وهو كريات الدم الحمر للبشر وثانيهما اختبار جلد الكتكوت حديث الفقس للتحري عن القابلية التشطيرية للخلايا التائية للطير.

تم ملاحظة نمطين في *K.pneumoniae* ونمطين في *E.coli* باستخدام LCL وPAL . ووجدت عزول من *K.pneumoniae* و*E.coli* غير منمطة . وتبين بان كل من LCL وPAL له وسع تنميطي شامل لكريات الدم الحمر للبشر ABH^+ وكان كل من LCL وPAL ذا وسع تشطيري للخلايا اللمفية التائية في الحي بنظام جلد جناح الكتكوت حديث الفقس . وبذلك يكون لكل من LCL و PAL وسع تنميطي للخلايا أولية النواة وحقيقية النواة ووسع تشطيري للخلايا اللمفية التائية للكتكوت حديث الفقس . ربما يعزى ذلك لتفاعل بروتين – بروتين او بروتين - كاربوهيدرات .

Introduction

Lectin are defined as a class of conjugated protein of non immune and non enzyme origin. That bind carbohydrates without modifying them . The term lectin was originally denoted to the materials that are soluble, multivalent glycoprotein can able of agglutinations with variety of cells(Sharon & Lis, 1990,2003) . Lectins are ubiqioutus in nature mapped in plants ,animals and microbes (Lis & Sharon, 1986) . In plants, however, they are mostly present in seeds (Goldstein & Poretz, 1986 ; Rudiger, 1993; Sharon & Lis ,2003) . In practice, plant lectins can be used in cell agglutination, blood typing, bacterial typing and mitogenesis of cells (Rudiger, 1997).

Mitogenesis can be tested by *in vitro* lymphocyte culture (Fudenberg *et al*.,1976) lymphoblast transformation with radioactive thymidin incorporation in DNA (Bradshow, 1996) as well as phytohaemagglutinin avian skin test (Smith *et al*.,1999) . The aims of the present study are at the investigation of typing and mitogenic potentials of two legium seed lectins through performing the following steps:

a-Extraction.

b-Separation.

c-Partial characterization.
d- Blood typing.
e-Bacterial typing.
f-Avian T-cell mitogenicity via lectin avian skin test.

Materials & Methods

1-Test lectins :

The test plant seed are the lentil *Lens cinarius* and the mung bean seed *phaseleus aureus* Roux (AL-Rawi & Chakravarty, 1988).The seeds were ;washed, dried , morted then milled by a coffee miller .The resulted mil was denoted as a powder . the powder, then used to prepare 10% cold , hot and alcoholic extracts Fig IA&B. The LCL and PAL were prepared as in the flow chart Fig:2(Shnawa, 2001modified from sharon 1976).

2-In vitro Procaryotic Cellular Test System :

A Loopfull transfer from fresh *E.coli* and *K.peumoniae* 5µ/µL suspension were made on the surface of clean glass slide . Then a drop of the test lectin solutions were added to the drops on the slides . Mix then leave 1-2 min at room temperature check for agglutination particals (Sharon, 1976) .

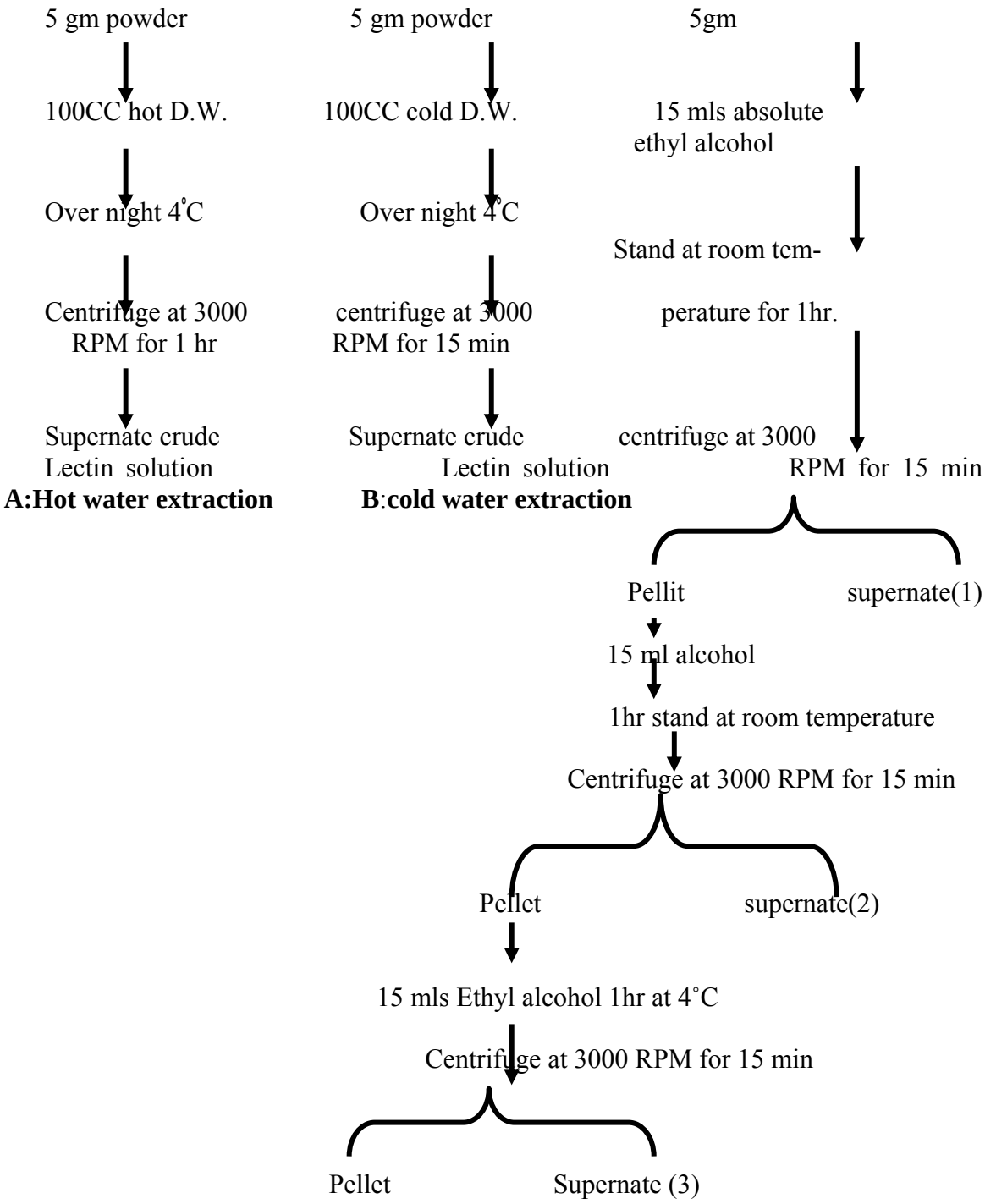
3-In vitro Eucaryotic cellular test system :

The blood typing was done as washed human erythrocyte suspensions of the groups A^+ , AB^+, H^+ were prepared as the test system. The serial dilution of 2,4,8,10……were prepared from the test lectins with size of 50µ in microhaemagglutination plate of 12 wells . 50 µL amounts of each erythrocyte type were added the sets of dilution test lectins . Erythrocyte lectin mixture in the test tries were incubated at 37°c for 45 min and checked for matt and bottoms . Then reincubated at 4° C for overnight rechecked for matt and bottoms .

Sugar binding assay (Shnawa & Abd, 2005; Kwapniski, 1972) were done as, sugar solution can be easly adsorped to erythrocyte membrane so 10% of glucose, maltose and galactose solutions were two fold diluted in microhaemagglutination try of 12 wells . Then, LCL and PAL coated sheep erythrocyte were added in 50 µL to each well in the dilution set of each sugar , in 50µL amounts . LCL and PAL coated erythrocyte-sugar dilutions mixtures were incubated at 37° C for 45 min and checked for matt and bottom then reincubated for overnight at 4°c and rechecked for matt and bottom formations .

4-In vitro avian T-cell mitogenicity test system :

Newly hatched brioler chicks were injected with 0.1 ml of the test lectins in the middle of the first wing web (patagium). In the second web , however, similar volume of sterile saline solution was injected in similar way as control. Standard Phytohaemagglutinin (PHA) was injected in other chick as positive control in one wing and 0.1ml of 1% ammonium sulfate in other wing to exclude the effect of residues inspite of performing dialysis (Kwapniski, 1972; Smith,1999).



In C 1,2&3 supernatants were collected . This is a crude lectin solution.

Fig1: *Phaseleus aureus* Lectin extraction

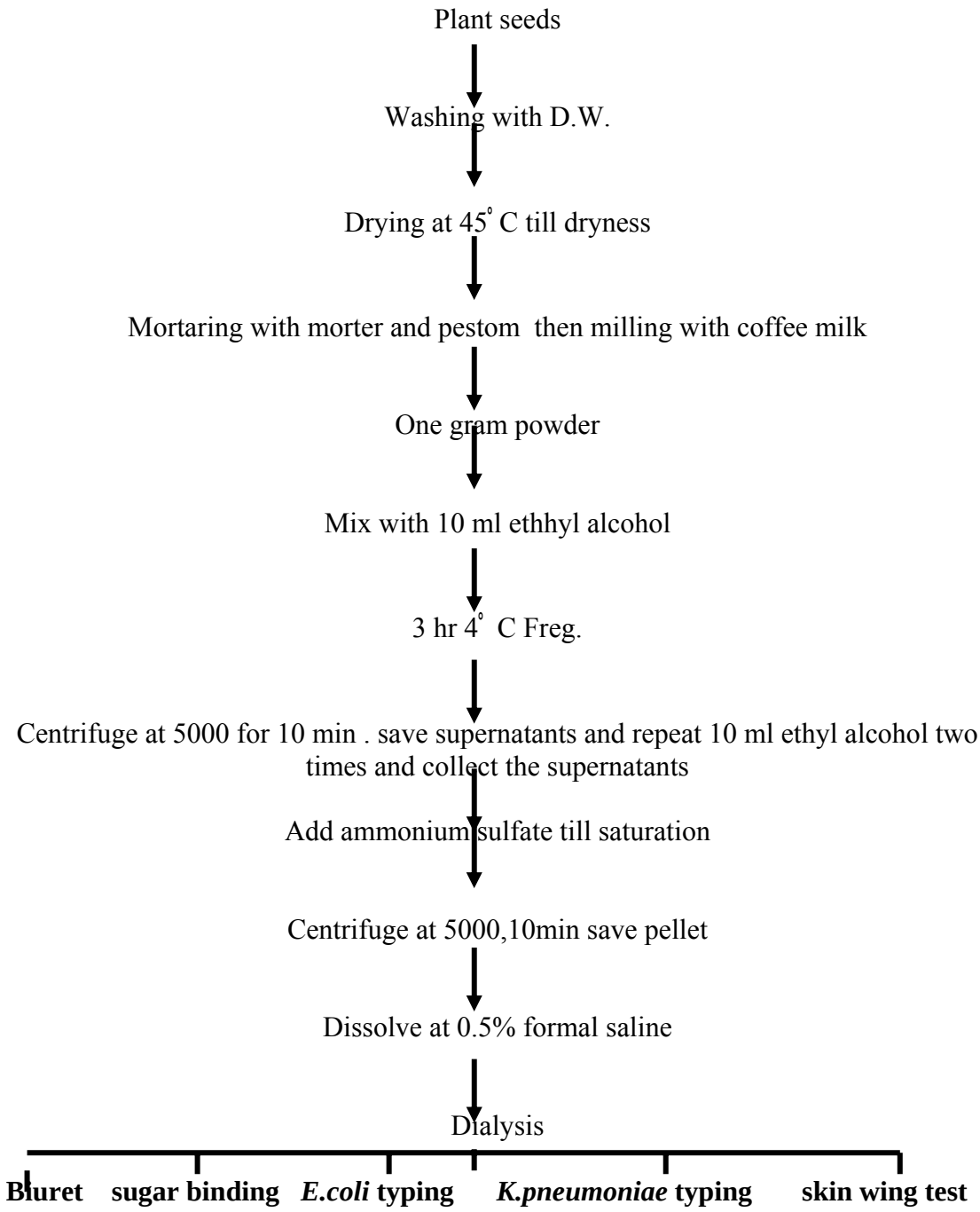


Fig.2:Extraction , separation and partial characterization for the test seed lectins.

Result

1- Partial characterization:

The preliminary experiment ,the phaselus aureus seeds were washed and deried . seeds were mortared and milled ,then were made in four makes ,powder, cold water, hot water and alcoholic extracts. The best supposed lectin containing is the alcoholic (Table 1).

The crud separat is colloidal solution ,biurate positive and precipitable by ammonium sulfate . Such crud separat is plant seed protein solution (Table 2).

Such plant seed protein solutions were able to agglutinate AB^+ whole human blood . They are able to bind glucose, maltose and galactose (Table3) .

2- In vitro prokaryotic cellular test system :

A- *E.coli* typing :

LCL typing 20:25 (80%) of the test *E.coli* isolated .The test rest 5:25 (20%) were untypeable . PAL typing all *E.coli* isolates.Two typing patterns were noted. (Table 4)

B- *Klebsiella pneumoniae* typing :

LCL type 12/18(66.6%) of the test *K. pneumoniae* isolate,leaving 6/18(33.3%) as untypable . PAL typed 18/18(100%) of the isolates thus ,two typing patterns are documented (Table4).

3-In vitro eukaryotic cellular test system :

LCL and PAL typed A^+,B^+,AB^+ and H^+ with quantitative haemagglutinin differences as indicated by the titre differences which ranges from 16-128. Thus LCL and PAL have the potential to be of
Pan agglutinating agents (Table5).

3- *In vivo* avian T-lymphocyte mitogenicity :

The skin thickness (induration) was 4.5 mm in LCL and 4.0mm in PAL in comparison to 2mm for standard PAL preparation and nill for 1% ammonium sulfate solution (Table 6).

Table1: Effect of extraction and separation methods on haemoagglutination nature of *P.aureus* Roux.

Seed preparation nature	Washed 2% erythrocyte of			
	A	B	AB	H
Powder	+D	+D	+D	-
Cold water	(+)4	-	-	-
Hot water	(+)4	(+)4	-	-
Alcoholic	(+)4	-	+8	++16

Table 2: Partial characterization of the test plant seed protein solutions.

1	Colloidal solution ,precipitable by ammonium sulfate
2	Yellowish in *L.cinarius* and Greenish in *P. aureus*
3	Biuret positive
4	Haemoagglutinate 1:5 AB^+ whole Blood

Table 3: Plant seed protein binding to carbohydrates.

Test lectin	Glucose	Maltose	Galactose
LCL	2.5	5	2.5
PAL	0.625	0.625	0.625

Table 4:*K.pneumoniae* and *E.coli* lectin typing patterns

Test bacteria	Agglutination with lectin of		
	LCL	PAL	Reaction patterns
K.pneumoniae	+	+	12:18(66.6%) I
	-	+	6:18(33.3%) II
E.coli	+	+	20:25(80%) I
	-	+	5:25(20%)II

Table 5:Human erythrocyte typing

Test lectin	2% Erythrocyte suspensions			
	A^+	B^+	AB^+	H
LCL	32	64	64	64
PAL	16	32	128	128

Table 6:*In vivo* avian T cell mitogenicity

Test lectin	Skin thickness before	Skin thickness after	Indurations/ mm
LCL	0.5	5	4.5
PAL	0.5	4.5	4
PHA	0.5	2.5	2
Standard ammonium sulfate (1%)	0.5	0.5	0

Discussion

Lectins are of major interest , since they act as recognition determinants in many biological processes such as adhesion of the infectious agents to host cells and cell-cell interactions in the immune system . Lectins had been shown perform key recognition functions in the immune system (Lis & Sharon, 1998) . Lectins are of non mitogenic and mitogenic types the later are polyclonal activator for lymphocyte (Sharon, 1976) . They are ubiquitous in nature and play a role in cancer, fertilization, immune response and signal transduction (Gabius& Gabius, 1997).

Lectins are proteins that bind carbohydrate in a reversible manner (Gabius & Gabius, 1997). They are mono, di , and multivalent types . the multivalent type cross linked complexes with multivalent carbohydrates epitopes which fall in to linear type II cross-links (Brewer, 1996; Hametryck *et al*.,2000) .

The legium lectins are widely used as a model system for studying protein-carbohydrate interaction, and protein- protein interactions (Buts *et al*.,2002). Several function are attributed to phytolectins such as ; cell separation, blood typing , bacterial typing , emberyonic and cancer cell agglutination, sperm-ovum interactions , fungal cell inhibition as well as lymphocyte mitogenicity (Sharon& Lis, 1990,2003 ; Rudiger, 1997).

In the present study a colloidal solutions from seed powders were separated using cold ethyl alcohol (Shnawa, 2001) Fig 2. and were precipitated by ammonium sulfate salting out (Shnawa, 2001),dialyzed (Sharon & Lis, 1990) , biurate positive ,agglutinate 1/5 AB^+ whole blood and bind carbohydrates,like glucose,maltose and galactose (Haq & Westwood, 2002) . Thus, they are partially identified plant seed

lectin solution (Shnawa, 2001). These LCL and PAL lectins showed panagglutinability with A^+,B^+,AB^+ and H^+ 2% washed human erythrocytes to variable titre limits of up to 128 (Sharon, 1976).

Phylolectin typing system serve as one of the known identification and epidemiologyic tools for screening of several bacterial genera like *Staphylococcus,Streptococcus,Compylobacter,Helicobacter,Lactobacillu, Salmonella* and *Listeria* (Hynes *et al*.,1999 ; Shnawa *et al*., 2004, table:7)

Bacterial lectin agglutination in the reaction mixture microenvironment stats as lectin molecule bind sugar residues attached to the cell surface receptors where by agglutination appeared when, large numbers of lectin molecules cross – links the sugar residues to these surface receptors (Sheeler & Bianch, 1983).These lectin typing findings can be considered as alternative to the serotyping systems when the lectin reacts with a carbohydrate structure which in some instances many coiencid with an epitope conferring serotype specifity (Gill & Corbel, 1986). Hence, it is reasonable to predict future tense application to the potential of lectins in typing of medical bacteria like *K . pneumoniae* and *E.coli* (Table 7).

Table 7:Bacterial lectin typing

Bacterial species	Lectin source	Lectin type/pattern	Ref.
H.pylori	AAA,ECA,LA,UEA,WGA	δ lectin pattern	Hynes *et al*., 1990
Cambylobacter	AHL,BpL,TrL,StL,WFL	23 lectin patterns	O`Sullivan *et al*., 1990
C.fetus type A	H.P.L	1Lectin type	Gill & Gorbal 1986
S.epidermidis	WGA,SBA,LCA, ConA,	25 patterns	Janor *et al*.,1992
S.aureus	Several, IE type	Several patterns	Munoz *et al*., 1999
Ed.ictluri	Ricinus	1 lectin patterns	Ainsworth, 1993
K.pneumoniae	OSL,CML,PML	2 patterns	Shnawa *et al*., 2004
K.pneumoniae	LCL,PAL	2 patterns	This study
E.coli	LCL,PAL	2 patterns	

In an *in vivo* avian T-lymphocyte mitogenicity test multivalency polyclonality and sugar binding potential lead to cross-link may be of type I and II (Brewer,1996 ; Hametryck *et al*.,2000) facilitating signal transduction change in metabolic turnover rate , mitogenic event which ends with lymphocyte proliferation, expention and migration as well as macrophage infiltration . These events are fallowed by perivascular lymphocyte accumulation due to lectin stimulation (Christi,2001; Smith,1999). Blood smear from lectin treated newly hatched chicks showed various mitotic figures (Sheeler & Bianch, 1983) . Thus LCL and PAL are mitogenic by lectin skin test in newly hatched chicks (Table 8) while there are *in vitro* methods for testing T-lymphocyte mitogenicity like direct lymphoblast count from lymphocyte culture (Shnawa & AL-Shahery , 2004) . radioactive thymidine incorporation during DNA synthesis and direct dye release (Smith,1999) . Thus ,on summing up the basic immunobiologic features of LCL and PAL one may point out :

1- Multiple protein species or multivalent protein.
2- Bind glucose , maltose and galactose .
3- Panghaemagglutinate ABH^{+} human erythrocytes.
4- Have reasonable *E.coli* and *K. pneumoniae* typing potential with predicted epidemiologic value.
5- Both LCL and PAL are T cell mitogene in an *in vivo* avian model.

Table 8 : PHA *in vivo* mitogenicity

Test system	Test lectin / con. mg	Skin Induration mm	Reference
Houce Martin	PHA 0.65Mg	2.98	Christi *et al*., 2001
Tree Swallow American Kistrel	PHA 40Mg	0.93	Smites *et al*., 1999
	50Mg	2.76	
Newly Hotched Briolyer Checkes	CM 5.565	1.6	Shnawa &AL-Byattes 2009
	CS 3.648	1.8	
	CV 1.673	1.2	
	CC 1.115	1.2	
	LCL 3.1	4.5	This study
	PAL 2.25	4.0	

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