A purified lectin with larvicidal activity from a woodland mushroom, *Agaricus semotus* Fr.

Isaiah O. Adedoyin¹, Taiwo S. Adewole², Titilayo O. Agunbiade³, Francis B. Adewoyin⁴, Adenike Kuku¹,*

¹Department of Biochemistry and Molecular Biology, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria.
²Department of Chemical Sciences, Kings University, Ode-Omu, Osun State, Nigeria.
³Department of Chemical Sciences, Oduduwa University, Ipetumodu, Osun State, Nigeria.
⁴Drug Research and Production Unit, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria.

**ABSTRACT**  This study investigated the larvicidal activity on *Culex quinquefasciatus* of lectin purified from fresh fruiting bodies of woodland mushroom, *Agaricus semotus*. *A. semotus* lectin (ASL) was purified via ion-exchange chromatography on DEAE-cellulose A-25 and size exclusion chromatography on Sephadex G-100 matrix. Molecular weight (16.6 kDa) was estimated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The effects of temperature, pH, metal chelation- and larvicidal activity of ASL were also investigated. The ASL indifferently agglutinated the erythrocytes of the human ABO blood system and was stable at acidic pH and below 50 °C whereas 66% of its activity was lost at 60 °C with complete inactivation at 70 °C. ASL is a metalloprotein requiring barium ion as chelation of metals by 50 mM EDTA rendered the lectin inactive, while the addition of BaCl₂ among other metal salts, restored the activity. ASL showed larvicidal activity against *C. quinquefasciatus* larvae after 24 h with a mortality of 5 and 95% at 5 and 25 mg/mL respectively, and LC₅₀ of 13.80 mg/mL. This study concluded that purified *A. semotus* lectin showed impressive larvicidal activity, which could be exploited in its development as an insecticidal agent.

**KEY WORDS**  
*Agaricus semotus*  
*Culex quinquefasciatus*  
larvicidal activity  
lectin

**ARTICLE INFORMATION**  
Submitted  
21 December 2020.  
Accepted  
17 February 2021.  
*Corresponding author  
E-mail: adenikekkuku@yahoo.com;  
akuku@oauife.edu.ng

**ARTICLE INFORMATION**

**Introduction**

Diseases transmitted by mosquitoes are one of the endemic health-related environmental menaces on the rise worldwide, which contribute to a high loss of life, especially in tropical countries with low income (Wilke et al. 2017; Fonseca et al. 2019). *Culex quinquefasciatus* Say, 1823 (*Culicidae*, southern house mosquito) is vastly distributed in subtropical regions of the world (Lopes et al. 2019). It is incursive, and belongs to the *Culex pipiens* species complex, which feed on mammalian and avian blood, *C. quinquefasciatus* is considered a medically significant species, because of their implicated roles in the transmission of zoonotic diseases including those caused by *Wuchereria bancrofti*, West Nile virus, and arbovirus (Fonseca et al. 2004; Lima-Camara et al. 2016).

Although majorly found in Africa, *C. quinquefasciatus* usually spread beyond their domiciled environment, posing a severe risk to public health (Farnesi et al. 2015; Cuthbert et al. 2020). As part of control measures to curb mosquito establishment and proliferation, the World Health Organization (WHO) introduced a manual on the management of these quintessential vectors (Takken and van den Berg 2019). Due to the advent of mosquito strains resistant to synthetic insecticides, together with potential environmental and health threats associated with these chemicals, alternative bio-control agents are being sought (Bellinato et al. 2016; Camaroti et al. 2017; Santos 2020).

The non-enzymatic lectins with unique sugar-binding characteristics are ubiquitous proteins capable of reversible discrete interaction with cell surface carbohydrate associated structures and involved in diverse biological and pathological functions (He et al. 2015; Muszyńska et al. 2018; Nascimento et al. 2020; Perduca et al. 2020). Aside from being a remarkable tool in blood group determination, lectins also perform defense-related functions (Silva et al. 2020). Although originally identified in plants and animals, exploration of fungal lectins is becoming popular because of their peculiar carbohydrate specificity coupled with potential application in biotechnology and biomedicine (Singh et al. 2010; Varrot et al. 2013; Hassan et al. 2015; Singh et al. 2015). Mushrooms have attracted numerous research activities due their diverse inherent bioactive constituents including lectins (Singh et al. 2010;
Largeteau et al. 2011).

Worldwide, *Agaricus* genus has over 300 members, which are distinguished by their unique spore coloration, among other structural features (Zhang et al. 2019). In this study a lectin was purified from the fresh fruiting bodies of woodland mushroom and its larvicidal activity was investigated towards *C. quinquefasciatus* to explore its potential in insect control.

**Materials and Methods**

**Materials and chemicals**

Glutaraldehyde, Folin reagent, ethylenediaminetetraacetic acid (EDTA), acrylamide, bovine serum albumin, sodium dodecyl sulfate (SDS), sugars, Coomassie Brilliant Blue R 250 and molecular weight protein markers (10-170 kDa) were purchased from Sigma-Aldrich (St Louis, Missouri, USA). Purchase of Sephadex G-100 and DEAE cellulose A-25 was from Pharmacia Fine Chemicals (Uppsala, Sweden). The quality of all reagents was research-quality.

A, B, O human blood groups were collected with informed consent from healthy subjects.

**Collection of mushroom**

Woodland mushroom (*A. semotus* Fries) was collected on farmland near the Department of Electrical and Electronics Engineering, Obafemi Awolowo University, Ile-Ife (South-West Nigeria). Identification was done at the Department of Microbiology (Mycology Laboratory), Obafemi Awolowo University, Ile-Ife, Nigeria.

**A. semotus crude extract preparation**

Approximately 16 g mushroom sample was homogenized and extracted using 0.025 M Phosphate Buffered Saline, pH 7.2 (1:5 w/v) and was stirred at 4 °C overnight. Resulting mixture was centrifuged at 4000 rpm for 15 min and filtered through cheesecloth to get the crude extract, which was stored at -20 °C until use.

**Protein concentration assay**

Assay method of Lowry et al. (1951) was employed in estimating protein concentration. Standard protein used was bovine serum albumin (1 mg/mL).

**Hemagglutination and sugar specificity assays**

Human A, B, and O blood groups were collected into lithium-heparin bottles. Erythrocytes were recovered from the centrifuged blood samples and fixed using glutaraldehyde (Bing et al. 1967). Hemagglutination assay and sugar specificity test was performed as described by Kuku and Eretan (2004) in a U-shaped 96-well microtiter plate. Tested sugars were D-mannitol, fructose, L-sorbose, galactose, D-mannose, D-glucosamine hydrochloride, N-acetyl-D-glucosamine, inulin, maltose, glycogen, D-lactose monohydrate, D-glucose monohydrate, and starch.

**Purification of A. semotus lectin (ASL)**

**Ion exchange chromatography on DEAE cellulose A-25**

Chromatography column (1.5 x 20 cm, Bio-Rad) packed with DEAE- cellulose A-25 matrix, and equilibrated with 10 mM Tris-HCl buffer, pH 7.3, was used to purify the crude protein extract (3 mL, ≈33 mg protein). Fractions (2 mL) were collected at 24 mL/h flow rate. Unadsorbed fractions were eluted with the equilibration buffer (10 mM Tris-HCl buffer, pH 7.3), while adsorbed fractions eluted with the same buffer containing 0.2 M NaCl. Fractions were monitored at 280 nm, and hemagglutinating activities were assayed. Active peak fractions (unadsorbed peak) were pooled, dialyzed (against 10 mM Tris-HCl buffer, pH 7.3, and distilled water) for 48 h, freeze-dried, and kept at -20 °C.

**Size exclusion chromatography on Sephadex G-100**

Five milliliters (≈ 30 mg protein) of the pooled DEAE active fractions (unadsorbed peak) was loaded on Sephadex G-100 column (2.5 x 20 cm, Bio-Rad) equilibrated with phosphate-buffered saline (0.02 M, pH 7.2), and fractions (4 mL) collected at 30 mL/h flow rate. Fractions were monitored at 280 nm, and hemagglutinating activity assayed. Active peak fractions were collected, dialyzed, freeze-dried, and kept frozen at -20 °C.

**Physicochemical characterization of ASL**

Denaturing gel electrophoresis (12.5% acrylamide gel, Tris-HCl system) was employed in the determination of ASL subunit molecular weight using standard marker proteins of 10-170 kDa range, as described by Laemmli

**Table 1. Purification summary.**

| Fractions                              | Total protein (mg) | Total activity (HU) | Specific activity (HU/mg) | Yield (%) | Purification fold |
|----------------------------------------|--------------------|---------------------|---------------------------|-----------|-------------------|
| Crude extract                          | 5550               | 1024                | 0.184                     | 100.0     | 1.00              |
| Ion exchange chromatography (IEC-1)    | 585.90             | 512                 | 0.873                     | 50.0      | 4.74              |
| Size exclusion chromatography (SEC-2)  | 101.8              | 128                 | 1.257                     | 12.5      | 6.83              |
Ion exchange and size exclusion chromatography purified ASL from *A. semotus* to homogeneity with yield and purification fold of 12.5% and 6.83, respectively (Table 1). Two distinct peaks were obtained from the elution profile of *A. semotus* crude extract on DEAE cellulose A-25 column (Fig. 1), of which only the unadsorbed peak (IEC-1) exhibited hemagglutinating activity. Size exclusion chromatography of the DEAE-active peak (IEC-1) on Sephadex G-100 matrix also resulted in two protein peaks (Fig. 2), but only second peak (SEC-2) showed hemagglutinating activity, which constituted the homogeneous ASL used for further studies.

Hemagglutination assay showed that ASL indifferently agglutinated the erythrocytes of the human ABO blood group, and this activity was strongly inhibited by inulin among tested sugars (Table 2).

**Larvicidal assay**

*C. quinquefasciatus* larvae were cultured in an insectarium under regulated conditions of relative humidity 70 ± 10% and at 27 ± 2 °C with a 12-12 light-dark regime at the Drug Research and Production Unit (DRPU), Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife. Free adult mosquitoes were allowed to lay eggs in the habitats provided for them in the laboratory and egg rafts collected for hatching in glass and plastic bowls. To promote larva development and female fecundity, hatched mosquito larvae were fed daily with fish food *ad libitum*.

Larvicidal activity of ASL was investigated as described in our previous study (Johnny et al. 2016). Laboratory-reared fourth instar larvae of *C. quinquefasciatus* were exposed to varying concentrations (5–25 mg/mL) of ASL according to standard procedure (WHO 1981). Comprehensively, 20 larvae were introduced into plastic beakers (50 mL) for each concentration of ASL. Experiments were done in triplicates at 27 ± 2 °C, with one control (distilled H₂O) set for each. Motionless larvae or those which settled at the bottom of test beakers with no sensitivity to involuntary stimulus or light, or after mild probing failed to move were considered dead. Percentage mortalities were recorded for each concentration after 24 h of the exposure period and obtained data statistically analyzed.

**Statistical analysis**

Experimental data were analyzed using StatPlus® 2006 (AnalystSoft, Canada) to find the lethal concentrations (LC₅₀) of larvae in 24 h by probit analysis with a reliability interval of 95% (Finney 1971; Islam et al. 2013).

**Results**

**Purification, hemagglutinating activity, and sugar specificity of Agaricus semotus lectin (ASL)**

Ion exchange and size exclusion chromatography purified ASL from *A. semotus* to homogeneity with yield and purification fold of 12.5% and 6.83, respectively (Table 1).

Two distinct peaks were obtained from the elution profile of *A. semotus* crude extract on DEAE cellulose A-25 column (Fig. 1), of which only the unadsorbed peak (IEC-1) exhibited hemagglutinating activity. Size exclusion chromatography of the DEAE-active peak (IEC-1) on Sephadex G-100 matrix also resulted in two protein peaks (Fig. 2), but only second peak (SEC-2) showed hemagglutinating activity, which constituted the homogeneous ASL used for further studies.

Hemagglutination assay showed that ASL indifferently agglutinated the erythrocytes of the human ABO blood group, and this activity was strongly inhibited by inulin among tested sugars (Table 2).

**Physicochemical characterization of ASL**

SDS-PAGE analysis of ASL revealed a distinct band with a relative molecular weight of 16.6 kDa (Fig. 3). ASL lost its activity at 70 °C (Fig. 4) and stable at acidic pH (Fig. 5).

**Larvicidal study**

The ASL treatment resulted the concentration-dependent mortality of *C. quinquefasciatus* larvae with the lowest mortality of 5% at 5 mg/mL and the highest mortality of
95% at 25 mg/mL concentration after 24 h (Table 3) with LC50 of 13.767 mg/mL (Fig. 6).

**Discussion**

**Purification, hemagglutinating activity and sugar specificity of Agaricus semotus lectin (ASL)**

Diverse chromatographic techniques are being used in the purification of mushroom lectins, however, recent studies have reported the effectiveness of ion-exchange and size exclusion chromatographic techniques as an alternative to highly preferred single-step affinity chromatography, which exploits sugar specificity of the lectin (Chandrasekaran et al. 2016; Wang et al. 2018; Panchak 2019). Elution profile of ASL on DEAE cellulose A-25 matrix reported in this study was similar to the chromatographic behavior of *A. arvensis* lectin (AAL) (Zhao et al. 2011).

*A. semotus* lectin (ASL) exhibited a non-specific agglutination towards the human ABO erythrocytes, characteristics mainly associated with an unique group of promiscuous lectins (panalectins), which have been identified mainly in plants, but rarely in mushrooms (Kuku et al. 2000; Akinyoola et al. 2016; Oladokun et al. 2019). However, lectins have been already identified and isolated within the genus *Agaricus*, there is not any previous report on the lectins of *A. semotus* (Kawagishi et al. 1988; Mikiashvili et al. 2006; Nakamura-Tsuruta et al. 2006; Zhang et al. 2019).

The hapten inhibition test to determine the sugar specificity of ASL showed that D-mannose, L-sorbose, galactose, and fructose slightly decreased the hemagglutinating activity, while inulin (a fructan) strongly inhibited the activity, although starch enhanced activity among the sugars tested (Table 2). Inhibition of the hemagglutinating activity of ASL by more than one simple and/or complex sugar reported in this study is not uncommon among mushroom lectins, especially within the genus *Agaricus*, as similar characteristics have been reported for lec-

![Figure 2. Size exclusion chromatography of the DEAE-Unadsorbed peak (IEC-1).](image)

| S/N | Concentration (mg/mL) | Number of larvae | Recorded death | Mortality % |
|-----|-----------------------|------------------|----------------|-------------|
| 1   | Control (distilled water) | 20.00            | 0.00 ± 0.00    | 0.00 ± 0.00 |
| 2   | 5.00                  | 20.00            | 1.00 ± 0.05    | 5.00 ± 0.12 |
| 3   | 10.00                 | 20.00            | 5.00 ± 0.25    | 25.00 ± 0.31 |
| 4   | 15.00                 | 20.00            | 8.00 ± 0.40    | 40.00 ± 0.11 |
| 5   | 20.00                 | 20.00            | 15.00 ± 0.75   | 75.00 ± 0.25 |
| 6   | 25.00                 | 20.00            | 19.00 ± 0.95   | 95.00 ± 0.11 |

Experiments comprised ASL (100 µL) diluted serially in a 96-well microtitre plate. Equal volumes (50 µL) of respective sugar solutions (0.2 M) and 2% human blood group A erythrocytes suspension were introduced to the wells. Positive control contained no sugars, and negative control contained neither ASL nor sugars. Experiments were done in triplicates.

Table 3. Larvicidal activity of ASL on *C. quinquefasciatus*.

![Table 2](image)

| Sugar                        | Hemagglutination titre |
|------------------------------|------------------------|
| Control                      | 2<sup>i</sup>          |
| Fructose                     | 2<sup>i</sup>          |
| L-Sorbose                    | 2<sup>i</sup>          |
| Galactose                    | 2<sup>i</sup>          |
| D-Mannose                    | 2<sup>i</sup>          |
| D-Mannitol                   | 2<sup>i</sup>          |
| D-Glucosamine-hydrochloride  | 2<sup>i</sup>          |
| D-Glucose monohydrate        | 2<sup>i</sup>          |
| N-acetyl-D-glucosamine       | 2<sup>i</sup>          |
| Inulin                       | 2<sup>i</sup>          |
| Maltose                      | 2<sup>i</sup>          |
| Glycogen                     | 2<sup>i</sup>          |
| D-Lactose monohydrate        | 2<sup>i</sup>          |
| Starch                       | 2<sup>i</sup>          |

Experiments were done in triplicates. Data were presented as mean ± SEM (standard error of mean).
tins isolated from *A. blazei*, *A. bisporus*, and *A. pilatianus* (Kawagishi et al. 1988; Nakamura-Tsuruta et al. 2006; Mikiashvili et al. 2006).

Inulin specificity obtained for ASL is similar to that of *A. arvensis* lectin (Zhao et al. 2011). Zhang et al. (2019) also reported inulin inhibition of *A. bitorquis* lectin suggesting a conserved inulin-binding specificity regarding isolated lectins from the genus *Agaricus*, which might be exploited in a structure-function relationship for practicable biotechnological application, especially through molecular docking. Other mushroom lectins reportedly inhibited by inulin include *Armillaria luteo-virens* lectin, *Pholiota adipose* lectin, and *Hericium erinaceum* lectin (Feng et al. 2006; Zhang et al. 2009; Li et al. 2010).

**Physicochemical characterization of ASL**

Fungal lectins, especially those isolated from mushrooms exhibit profound variation in their physicochemical properties (Hassan et al. 2015; Singh et al. 2020). The molecular weight of ASL (16.6 kDa) is similar to the weight reported for *A. blazei* lectin (16 kDa) (Kawagishi et al. 1988). ASL maintained 100% activity up to 50 °C, however, lost 66% of the activity at 60 °C and completely inactivated at 70 °C (Figure 4). ASL is moderately thermostable, as mushroom lectins are reportedly usually stable at a moderate temperature with gradual loss of activity as the temperature increases (Alborés 2014; Wang et al.)
2019). However, this result contradicts with the study of Zhao et al. (2011), who reported A. arvensis lectin was thermostable up to 90 °C. Several reports have shown that the thermostability of lectins differs, as lectins usually undergo unprecedented conformational changes under harsh temperatures, which might culminate in inactivation mostly owing to the destabilization of interactions necessary for their native conformation (Wang et al. 1996; Singh and Saxena 2013). ASL was stable at acidic pH with maximum activity within pH 2–5, with a 41% loss of activity within pH 7-9 and 50% loss at pH 11 (Fig. 5). Stability of ASL at acidic pH shown was similar to that reported for Gymnopilus spectabilis lectin (Alborés et al. 2014).

ASL was completely inactivated by 50 mM EDTA, however, when different metal salts were added to the assay medium, BaCl2, restored 33% of the lectin activity suggesting ASL might be a metalloprotein. The result obtained for ASL contradicts the report on A. arvensis lectin (AAL), which was unaffected by divalent ions and enhanced by trivalent ions (Zhao et al. 2011).

Larvicidal study

Chemical method of insect control is one of the major mechanisms of mitigating diseases propagated by these organisms with long-term effects linked with insect resistance, together with detrimental effects on the ecosystem, and human health, paving way for exploration of safer and eco-friendly natural alternatives such as lectins (Gautam et al. 2013; Shaurub et al. 2015; Demok et al. 2019; Satoto et al. 2019; Kumar et al. 2020).

As a distinctive component of the innate defense system of their host, mushroom lectins have a unique ability to recognize diverse carbohydrate-associated structures that mediate most of their varying biological activities (Singh and Saxena 2013; Sabotić et al. 2016). Previous studies on entomotoxic lectins have suggested the fatality induced by these proteins usually involves multiple complementary mechanisms including induction of apoptosis and interaction with specific carbohydrates/glycan structures of vital enzymes, especially those engaged in metabolism and detoxification (de Oliveira et al. 2016; Napoleão et al. 2018). In fact, because of their multivalent assembly, fungal lectins can cross-link cell surface glycoconjugates or trigger distinct oligomerization of glycosylated signaling receptors, which may affect their turnover (Hamshou et al. 2012). Morphological damages of the gut initiated by excessive proteolysis due to specific binding to peritrophic matrices or epithelial cells of target insects have also been connected to their insecticidal/larvicidal activity (Coelho et al. 2009). As reported by Coelho and co-workers, Moringa oleifera lectin increased lumen volume and disrupted the midgut epithelium of Aedes aegypti larvae and peritrophic membrane of Anagasta kuehniella larvae, ultimately resulting in gut cell death (Coelho et al. 2009; de Oliviera et al. 2017).

Entomotoxic lectins have also been reported to alter critical target insects’ biological functions such as pupation, survival, larval development, and adult emergence (Kaur et al. 2009). Dioscorea batatas lectin was reported to alter the development of Helicoverpa armigera larvae by interacting strongly with vital intracellular structures (Ohizumi et al. 2009).

The entomotoxic lectins vary in physicochemical characteristics, and there are many potential targets because of their multivalent highly stereospecific binding with diverse arrays of insect glycan structures, however, differences in their spatial arrangement together with the proportion of carbohydrate recognition domain may expound the disparity in toxicity mechanisms elicited by these quintessential bioactive proteins (Fitches et al. 2012; Yang et al. 2014; Rani et al. 2017; Singh et al. 2020).

Conclusion

This study concluded that a lectin purified from A. semotus showed potent larvicidal activity against C. quinquefasciatus, which suggests its application as an alternative and eco-friendly larvicide/insecticide in the control of mosquitoes to mitigate mosquitoes-borne diseases and mortalities. However, a better comprehension of the action mechanism used by the lectin might help further research to investigate the possibilities of biotechnological applications of mushroom lectins in agriculture against phytophagous insects.

References

Akinyoola KA, Odekanyin OO, Kuku A, Sosan MB (2016) Anti-insect potential of a lectin from the tuber, Dioscorea mangenotiana towards Eldana saccharina (Lepidoptera: Pyralidae). J Agric Biotechnol Sustain Dev 8(3):16-26. Alborés S, Mora P, Bustamante MJ, Cerdeiras MP, Fraguas LF (2014) Purification and applications of a lectin from the mushroom Gymnopilus spectabilis. Appl Microbiol Biotechnol 172:2081-2090. Bellinato DF, Viana-Medeiros PF, Araújo SC, Martins AJ, Lima JBR Valle D (2016) Resistance status to the insecticides temephos, deltamethrin, and diflubenzuron in Brazilian Aedes aegypti populations. BioMed Res Int 8603263:1-12. Bing DH, Weyand JGM, Stavitsky AB (1967) Hemagglutination with aldehyde-fixed erythrocytes for assay of antigens and antibodies. Proc Society Exp Biol Med
Lectin with larvicidal activity from Agaricus semotus

Coelho JS, Santos NDL, Napoleão TH, Ferreira RS, Zingali RB, Coelho LCBB, Leite SP, Navarro DMDAF, Paiva PMG (2009) Effect of Moringa oleifera lectin on development and mortality of Aedes aegypti. Chemosphere 77:934-938.

de Oliveira APS, de Santana Silva LL, de Oliveira CFR, de Moura MC, Navarro DMDAF, Zingali RB, Napoleão TH, Paiva PMG (2016) Biotechnological value of Moringa oleifera seed cake as source of insecticidal lectin against Aedes aegypti. Process Biochem 51(10):1683-1690.

demok S, Endersby-Harshman N, Vinit R, Timinao L, Rob-inson LJ, Susapi U, Makita L, Laman M, Hoffmann A, Karl S (2019) Insecticide resistance status of Aedes aegypti and Aedes albopictus mosquitoes in Papua New Guinea. Parasit Vec 12:333.

Farnesi LC, Menna-Barreto RFS, Martins AJ, Valle D, Rezense GL (2015) Physical features and chitin content of eggs from the mosquito vectors Aedes aegypti, Anopheles aquasalis and Culex quinquefasciatus: Connection with distinct levels of resistance to desiccation. J Ins Physiol 83:43-52.

124(4):1166-1170.

Camaroti JRSL, Oliveira APS, Paiva PMG, Pontual EV, Napoleão TH (2017) Phytoinsecticides for controlling pests and mosquito vectors of diseases. In Green V, Ed., Biocontrol Agents: Types, Applications and Research Insights. Nova Science Publishers, New York, 147-188.

Chandrasekaran G, Lee YC, Park H, Wu Y, Shin HJ (2016) Antibacterial and antifungal activities of lectin extracted from fruiting bodies of the Korean cauliflower medicinal mushroom, Sparassis latifolia (Agaricomycetes). Int J Med Mush 18(4):291-299.

Cuthbert RN, Cunningham EM, Crane K, Dick JTA, Cal-laghan A, Coughlan NE (2020) In for the kill: novel bios-eurity approaches for invasive and medically important mosquito species. Mang Biol Invasions 11(1):9-25.

de Oliveira APS, de Santana Silva LL, de Albuquerque Lima T, Pontual EV, de Lima Santos ND, Coelho LCBB, Navarro DMDAF, Zingali RB, Napoleão TH, Paiva PMG (2016) Biotechnological value of Moringa oleifera seed cake as source of insecticidal lectin against Aedes aegypti. Process Biochem 51(10):1683-1690.

demok S, Endersby-Harshman N, Vinit R, Timinao L, Rob-inson LJ, Susapi U, Makita L, Laman M, Hoffmann A, Karl S (2019) Insecticide resistance status of Aedes aegypti and Aedes albopictus mosquitoes in Papua New Guinea. Parasit Vec 12:333.

Farnesi LC, Menna-Barreto RFS, Martins AJ, Valle D, Rezense GL (2015) Physical features and chitin content of eggs from the mosquito vectors Aedes aegypti, Anopheles aquasalis and Culex quinquefasciatus: Connection with distinct levels of resistance to desiccation. J Ins Physiol 83:43-52.

Feng K, Liu QH, Ng TB, Wang HX (2006) Isolation and characterization of a novel lectin from the mushroom Armillaria luteo-virens. Biochem Biophys Res Commun 345:1573-1578.

Finney DJ (1971) Probit analysis. 3rd Ed., Cambridge University Press, New York.

Fitches EC, Pyati P, King GF, Gatehouse JA (2012) Fusion to snowdrop Lectin magnifies the oral activity of insectici-dal v-Hexatoxin-Hv1a peptide by enabling its delivery to the central nervous system. PLoS One 7(6):e39389.

Fonseca DM, Keyghobadi N, Malcolm CA, Mehmet C, Schaffner F, Mogi M, Fleischer RC, Wilkerson RC (2004) Emerging vectors in the Culex pipiens complex. Sci 303(5663):1535-1538.

Fonseca V, Xavier J, de Oliveira T, de Filippis AMB, Acantara LCJ, Giovanetti M (2019) Mosquito-borne viral diseases: control and prevention in the genomics era. In Curr T Epidemiol Vector-Borne Dis IntechOpen.

Gautam K, Kumar P, Poonia S (2013) Larvicidal and GC – MS analysis of flavonoids of Vitea negundo and Andrographis paniculata against two vector mosquitoes Anopheles ste-phenisi and Aedes aegypti. J Vec B Dis 50:171-178.

Hamshou M, van Damme EJ, Vandenborre G, Ghesquiere B, Trooskens G, Gevaert K, Smagghe G (2012) GalNAC/ Gal-binding Rhizoctonia solani agglutinin has antipro-liferative activity in Drosophila melanogaster S2 cells via MAPK and JAK/STAT signaling. PLoS ONE 7(4):e33680.

Hassan MAA, Rouf R, Tiralongo E, May TW, Tiralongo J (2015) Mushroom lectins: specificity, structure and bioactivity relevant to human disease. Int J Mol Sci 16(4):7802-7838.

He S, Shi J, Walid E, Zhang H, Ma Y, Xue SJ (2015) Reverse micellar extraction of lectin from black turtle bean (Phaseolus vulgaris): Optimisation of extraction conditions by response surface methodology. Food Chem 166:93-100.

Islam MS, Saiful M, Hossain M, Sikder M, Morshed M, Hossain M (2013) Acute toxicity of the mixtures of grease and engine wash oil on fish, Pangasius sutchi under laboratory condition. Int J Life Sci Biotech Pharm Res 2(1):306-317.

Johnny II, Kuku A, Odekanoyin OO, Adesina SK (2016) A lectin with larvicidal potential from the fresh leaves of Agelanthus brunnneus (Engl.) Van Tiegh Loranthaceae. J Pharm Res Int 13(3):1-9.

Kaur M, Singh K, Rup PJ, Kamboj SS, Singh J (2009) Anti-insect potential of lectins from Arisaema species towards Bactrocera cucurbitae. J Environ Biol 36:1263-1268.

Kawagishi H, Nomura A, Yumen T, Mizuno T, Hagiwara T, Nakamura T (1988) Isolation and properties of a lectin from fresh leaves of Agaricus blazei Murill. Process Biochem 23:1573-1578.

Kuwana M, Nomura A, Yumen T, Mizuno T, Hagiwara T, Nakamura T (1988) Isolation and properties of a lectin from fresh leaves of Agaricus blazei Murill. Process Biochem 23:1573-1578.

Lectin with larvicidal activity from Agaricus semotus

Kuku A, Eretan OB (2004) Purification and partial charac-terization of a lectin from the fresh leaves of Kalanchoe crenata (Andr.) Haw. BMB Rep 37(2):229-233.

Kuku A, Stoppani M, Cobianchi A, Minetti G, Baldunni C, Aboderin A (2000) The complete primary structure of a mannose/glucose specific lectin from the seeds of Dioscorea reflexa (Hook, F.). Nig J Biochem Mol Biol 15:115-119.

Kumar D, Kumar P, Singh H, Agrawal V (2020) Biocontrol of mosquito vectors through herbal-derived silver nanoparticles: prospects and challenges. E Sci Pol Res 27(21):25987-26024.

Laemmli UK, Favre M (1970) Maturation of the head of bacteriophage T4. I. DNA packaging events. J Mol Biol 71
A novel lectin inhibitory activities from dried fruiting bodies of the monkey head mushroom *Hericium erinaceum*. BioMed Res Int 2010:716515.

Lima-Camara TN (2016) Emerging arboviruses and public health challenges in Brazil. Rev Saude Publica 50:36.

Lopes RP, Lima JPB, Martins AJ (2019) Insecticide resistance in *Culex quinquefasciatus* Say, 1823 in Brazil: a review. Parasites Vectors 12(1):591.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193(1):265-275.

Mikashvili NA, Elisashvili V, Wasser SP, Nevo E (2006) Comparative study of lectin activity of higher Basidiomycetes. Int J Med Mush 8(1):31-38.

Muszyńska B, Grzywacz-Kisielewska A, Kala K, Gdula-Szymańska B, Varrot A, Basheer SM, Imberty A (2013) Fungal lectins: Structure, function and potential applications. Curr Opin Struct Biol 23(5):678-685.

Perduca M, Desteefanis L, Bovi M, Galliano M, Munari F, Assfalg M, Ferrari F, Monaco HL, Capaldi S (2020) Structure and properties of the oyster mushroom (*Pleurotus ostreatus*) lectin (POL). Glycobiology 30(8):550-562.

Rani S, Sharma V, Hada A, Bhattacharya RC, Koundal KR (2017) Fusion gene construct preparation with lectin and protease inhibitor genes against aphids and efficient genetic transformation of *Brassica juncea* using cotyledons explants. Acta Physiol Plant 39(5):115.

Sabotič J, Ohm RA, Künzler M (2016) Entomotoxic and nematotoxic lectins and protease inhibitors from fungal fruiting bodies. Appl Microbiol Biotechnol 100(1):91-111.

Sampaio LA, Tesser M, Pickersgill AR (1998) Temperature effects on sex differentiation of two South American atherinids, *Odontesthes argentinensis* and *Patagonina hatchery*. Environ Biol Fish 47:624-629.

Santos NDL, Napoléon TH, Benevides CA, Albuquerque LP, Pontual EV, Oliveira APS, Coelho LCBB, Navarro DMAF, Paiva PMG (2020) Effect of gamma irradiation of *Moringa oleifera* seed lectin on its larvicidal, ovicidal, and oviposition-stimulant activities against *Aedes aegypti*. Afr J Bot 129:3-8.

Satoto TBT, Satrisno H, Lazzuardi L, Diptyanusa A (2019) Insecticide resistance in *Aedes aegypti*: an impact from human urbanization? PLoS One 14(6):e0218079.

Shaurub EH, Abd El-Aziz NM (2015) Biochemical effects of lambda-cyhalothrin and lufenuron on *Culex pipiens* L (Diptera: Culicidae). Int J Mosq Res 2:122-126.

Silva AJ, Cavalcanti VLR, Porto ALF, Gama WA, Brandão-Costa, RMP, Bezerra RP (2020) The green microalgae *Tetradesmus obliquus* (Scenedesmus acutus) as lectin source in the recognition of ABO blood type: purification and characterization. J Appl Physiol 1-8.

Singh AP, Saxena KD (2013) Biological activity of purified *Momordica charantia* lectin. Chem Sci Trans 2:258-262.

Singh RS, Bhari R, Kaur HP (2010) Mushroom lectins: current status and future perspectives. Crit Rev Biochem 30(2):99-126.

Singh RS, Thakur SR, Bansal P (2015) Algal lectins as promising biomolecules for biomedical research. Crit Rev Microbiol 41:77-88.

Singh RS, Walia AK, Kennedy JF (2020) Mushroom lectins in biomedical research and development. Int J Biol Macromol 151:1340-1350.

Takken W, van den Berg H (2019) Manual on prevention of establishment and control of mosquitoes of public health importance in the WHO European Region. Copenhagen: World Health Organization.

Varrot A, Basheer SM, Imbery A (2013) Fungal lectins: structure, function and potential applications. Curr Opin Struct Biol 23(5):678-685.

Wang HX, Liu WK, Ng TB, Ooi VEC, Chang ST (1996) The immunomodulatory and antitumor activities of lectins
from the mushroom *Tricholoma mongolicum*. Immuno-
pharmacol 31:205-211.

Wang Y, Wua B, Shaob J, Jiaa J, Tianaa Y, Shuaa X, Renaa X, 
Guanaa Y (2018) Extraction, purification and physico-
chemical properties of a novel lectin from *Laetiporus 
sulphureus* mushroom. LWT Food Sci Technol 91:151-159.

Wilke ABB, Medeiros-Sousa AR, Ceretti-Junior W, Marrelli 
MT (2017) Mosquito populations’ dynamics associated 
with climate variations. Acta Trop 166:343-350.

WHO (1981) World Health Organizations. Instruction for 
determining the susceptibility or resistance of mos-
quitos’s larvae to insect development inhibitors. (WHO/ 
VBC/81.812). Geneva, 1-6.

Yang S, Pyati P, Fitches E, Gatehouse J (2014) A recombinant 
fusion protein containing a spider toxin specific for the 
insect voltage-gated sodium ion channel shows oral 
toxicity towards insects of different orders. Biochem 
Mol Biol 47(100):1-11.

Zhang GQ, Chen QJ, Hua J, Liu ZL, Sun Y, Xu X, Han P, 
Wang HX (2019) An inulin-specific lectin with anti-
HIV-1 reverse transcriptase, antiproliferative, and mi-
togenic activities from the edible mushroom *Agaricus 
bitorus*. Biomed Res Int 2019:1341370.

Zhang GQ, Sun J, Wang HX, NG TB (2009) A novel lec-
tin with antiproliferative activity from the medicinal 
mushroom *Pholiota adipose*. Acta Biochim Pol 56:415-421.

Zhao JK, Zhao YC, Li SH, Wang HX, Ng TB (2011) Isola-
tion and characterization of a novel thermostable lectin 
from the wild edible mushroom *Agaricus arvensis*. J Basic 
Microbiol 51(3):304-311.
