Pesticidal and pest repellency activities of a plant derived triterpenoid 2α,3β,21β,23,28-penta hydroxyl 12-oleanene against *Tribolium castaneum*

Alam Khan1*, Md Shariful Islam2, Moizur Rahman2, Tanjeena Zaman3 and Md Ekramul Haque1

**Abstract**

**Background:** *Tribolium castaneum* (Herbst) is a major pest of stored grain-based products, and cause severe damage to cereal grains throughout the world. The present investigation was aimed to determine the pesticidal and pest repellent activities of 2α,3β,21β,23,28-penta hydroxyl 12-oleanene against *T. castaneum*. The compound 2α,3β,21β,23,28-penta hydroxyl 12-oleanene is a triterpenoid which was isolated from the roots of *Laportea crenulata* Gaud. Surface film technique was used for pesticidal screening, whereas, pest repellency property of the triterpenoid was determined by filter paper disc method.

**Results:** At 24 hours of exposure duration, significant mortality records (80% and 86%) were observed at doses 0.88 and 1.77 mg/cm². No significant change in mortality records was observed when duration of exposure was increased up to 48 hours. The triterpenoid showed significant repellency activity at doses 0.47 and 0.94 mg/cm².

**Conclusion:** These data suggest that the triterpenoid 2α,3β,21β,23,28-penta hydroxyl 12-oleanene possess both pesticidal and pest repellency activities against *T. castaneum* and can be used in controlling the pest of grain-based products.

**Keywords:** *Laportea crenulata*, 2α,3β,21β,23,28-penta hydroxyl 12-oleanene, Botanical pesticide, Botanical pest repellent, *Tribolium castaneum*

**Background**

The red flour beetle, *Tribolium castaneum* (Herbst) is a primary pest of flour and other milled products of cereals, and a secondary pest of stored wheat, causing severe damages to these food grains by both quantity and quality [1-4]. A number of synthetic agents (e.g. methoprene, permethrin, cypermethrin, deltamethrin and fenvalerate etc.) were identified for good activity against *T. castaneum* and have been used to control the pest [4,5]. However, use of synthetic agents has led to a number of problems such as environmental disturbances, increasing costs of application, pest resurgence, pest resistance to pesticides, lethal effects on non-target organisms and direct toxicity to users [6]. Dyte and Blackman ([7]) reported that almost all of the strains of *T. castaneum* have become resistant to malathion. In recent years, there has been increasing information on the use of alternative methods [8], and plant extracts are the most commonly tested alternative products [9-14]. Different biological activities of plant derivatives were demonstrated for the control of stored-grain pests [11,15]. Plant products having considerable insecticidal potential are gaining remarkable importance in recent years because of minimizing the disadvantages associated with synthetic agents [16,17]. Certain plant families, particularly plant products of Rutaceae and Myrtaceae had shown, in previous observations, repellent, insecticidal, anti-feedant, and growth regulatory properties against insect pests of stored commodities [18-24]. Some of the Citrus plant species (*C. sinensis, C. aurantifolia, C. reticulata, C. limon*) (Rutaceae) have been also reported as a source of botanical insecticides [24-26]. A variety of these plants contain secondary metabolites that show insecticidal activity against several coleopteran and dipterans species [27,28].

*Laportea crenulata* Gaud. (Synonym *Urtica crenulata* Roxb.) is an evergreen shrub of Urticaceae family [29].
The Urticaceae are monoecious or dioecious herbs or infrequently shrubs or small trees comprising 45 genera and 700 species, often with specialized stinging hairs [30]. About 9 genera and 60 species are available in Bangladesh [31]. In Bangladesh, L. crenulata locally known as Agnichutra, also distributed in India and Malay island [29]. The roots are used traditionally for the treatment of bleeding from nose and/or mouth, excessive gas in the stomach, constipation, weakness, asthma, gout, mumps, whooping cough, and chronic fever [32]. The roots of the plant have stimulant, stomachic and diuretic properties [32]. Such traditional uses suggesting the presence of biologically active substances in roots of the plant. A triterpenoid 2α,3β,21β,23,28-penta hydroxyl 12-oleanene was isolated from the roots of the plant [33]. Searching botanical agent(s) to control T. castaneum, the present study aimed to determine the insecticidal and insect repellency activities of 2α,3β,21β,23,28-penta hydroxyl 12-oleanene against T. castaneum.

Results
The compound 2α,3β,21β,23,28-penta hydroxyl 12-oleanene at 24 hours duration of exposure, has exerted significantly high mortality records 80.00% and 86.60% at doses 0.88 and 1.77 mg/cm², respectively (Table 1; Figure 1). The compound showed moderate mortality (63.30%) at dose 0.44 mg/cm² and weak mortality (33.30%) at dose 0.22 mg/cm²; both were not significant for pesticidal uses. The higher mortality records with higher doses of the compound suggesting its dose dependent pesticidal activity against T. castaneum. When exposure duration was increased 24 to 48 hours, the mortality records were minutely increased for doses 0.22, 0.88 and 1.77 mg/cm², however, for 0.44 mg/cm² dose no change in mortality was observed (Table 2; Figure 1). Median lethal doses (LD₅₀) of the compound for 24 and 48 hours exposure duration were 0.38 and 0.34 mg/cm², respectively (Table 1).

At the beginning of exposure (1 h), moderate pest repellency activity (53.2 and 66.6% repulsion) was observed for doses 0.47 and 0.94 mg/cm², respectively (Table 3; Figure 2). Within the second hour, repellency records were sufficiently increased for all doses. At subsequent 3rd and 4th hours observation repellency records were little increased, but no increment was observed after 4th hour (Table 3). However, repellency activity at the lowest dose 0.23 mg/cm² always found insufficient. Overall, significant pest repellency activity for the triterpenoid was observed at 0.47 and 0.94 mg/cm².

Discussion
Insects/Pests are a problem in stored grain throughout the world, and cause serious losses in weight and quality of the stored products [17,34]. T. castaneum is a major pest of most grain, widely spread worldwide and very destructive [5,16]. As widely used synthetic insecticides/pesticides causing concern because of environmental, economy, resistance and toxicity issues, scientists are searching for alternative methods [6,8]. In many regions of the world, locally available materials are used to protect stored products against damage by pest infestation. Insecticides of plant origin, because of their high degree of tolerance by the mammals, are particularly desired for application against pests of fodders, fruits, vegetables and stored grains [35]. Moreover, interest in botanical insecticides has increased as a result of environmental concerns, and insect populations becoming resistant to conventional chemicals. The using of plant extracts in insect control has been practiced for at least two millennia, when botanical insecticides were considered important products for insect management in Ancient China, Egypt, India, Greece [5,6,36]. Moharrammipour et al. [37] and Shahkarami et al. [38] demonstrated that Ferula asaftetida L. extract and the essential oil of Artemisia aucheri Boiss. had antifeedant property on T. castaneum adults.

Triterpenoid 2α,3β,21β,23,28-penta hydroxyl 12-oleanene was isolated from roots of L. crenulata, has been shown both insecticidal and insect repellency properties against T. castaneum. The control of pest in stored food products can

| Table 1 LD₅₀ calculation for pesticidal activity using probit analysis |
|-----------------|----------------|----------------|----------------|----------------|----------------|
| Recording time  | Dose (mg/cm²) | % of Average mortality | % Corrected mortality | Regression equation | LD₅₀ (mg/cm²) | 95% Confidence limits |
|-----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| Record after 24 hours | 1.77 | 86.60 | 87 | Estimate 1 | 0.38 | 6.58 | 16.32 |
|                  | 0.88 | 80.00 | 80 | Estimate 2 | 1.92 | 1.58X |
|                  | 0.44 | 63.30 | 63 |             | 3.23 | 1.31X |
| Record after 48 hours | 1.77 | 90.00 | 90 | Estimate 1 | 0.34 | 9.54 | 22.55 |
|                  | 0.88 | 86.60 | 87 | Estimate 2 | 1.62 | 1.64X |
|                  | 0.44 | 63.30 | 63 |             | 3.23 | 1.31X |
be achieved by insecticidal and insect repellency activities [4,12,16,39]. Excellent pesticidal activity for the triterpenoid has been found at doses 0.88 and 1.77 mg/cm², whereas, sufficient pest repellency activity was observed at doses 0.47 and 0.94 mg/cm². Identification of pesticidal compounds in plant is not new; Luo et al. [40] isolated triptolide, triptonide and euonine from root barks of Tripterygium wilfordii Hook and confirmed activity against Mythimna separate. Pungitore et al. [41] identified oleanolic acid, maslinic acid, and daucosterol from Junellia aspera (Verbenaceae) and confirmed their toxic effect against T. castaneum. However, only a small number of pest control products directly obtained from plants [19,42]. Botanicals used as insecticides presently constitute only 1% of the world insecticide market [43]. In the present study, we confirmed toxic effect of the triterpenoid against T. castaneum. However, further investigations are necessary to know toxicity of the compound to mammals as well as to know the effect on nutritional status of grain.

**Conclusions**

The compound 2α,3β,21β,23,28-penta hydroxyl 12-oleanene has induced significant pesticidal activity against T. castaneum at doses 0.88 and 1.77 mg/cm². In contrast, significant pest repellency against the pest was observed at doses 0.47 and 0.94 mg/cm². In the light of the findings of present study, it can be stated that good pesticidal and pest repellency activities of 2α,3β,21β,23,28-penta hydroxyl 12-oleanene against T. castaneum suggesting its suitability as botanical pesticide in controlling the pest of stored grains and grain products.

**Methods**

**Plant materials**

The roots of L. crenulata was collected from various part of Rangpur district of Bangladesh in the month of October to November. Taxonomically the plant was identified by Professor A. T. M. Naderuzzaman, Department of Botany, University of Rajshahi, Rajshahi, Bangladesh and a voucher specimen (No. 1239) had been deposited in the Department.

The fresh roots was first washed with water to remove adhering dirt, cut into small pieces, sun dried for three days and finally dried at 45°C for 36 h in an electrical oven [33]. The dried roots were pulverized into a coarse powder [44] with the help of a grinding machine (FFc-15, China) and were stored in an air tight container for further use.

**Isolation of 2α,3β,21β,23,28-penta hydroxyl 12-oleanene**

Dried powdered roots (900 g) of the plant were extracted (cold) with ethanol (5 L) in flat bottom glass containers, through occasional shaking and stirring for 10 days. The whole extract was filtered and the solvent was evaporated to dryness in vacuo with an rotary evaporator at 40–50°C to afford a blackish green mass (45 g), which was further extracted with petroleum ether (3×50 mL), chloroform (3×50 mL), and methanol (3×50 mL) to afford petroleum ether (3 g), chloroform (7 g), and methanol (9 g) fractions, respectively [45,46].

The petroleum ether soluble fraction (5 g) was subjected to column chromatography using n-hexane, chloroform and methanol of increasing polarity. Column chromatography fractions eluting with 100% chloroform to 40% methanol in chloroform was subjected to preparative TLC.

| Dose (mg/cm²) | Mortality record for applied pests | Average ± SD | Record after 48 hours | Average ± SD |
|---------------|-----------------------------------|--------------|-----------------------|--------------|
| 1.77          | 10                                | 7            | 8.66 ± 1.52           | 8            | 8.66 ± 1.52 |
| 1.77          | 10                                | 9            |                       |              |              |
| 1.77          | 10                                | 10           |                       |              |              |
| 0.88          | 10                                | 8            | 8.00 ± 1.00           | 10           | 8.66 ± 1.52 |
| 0.88          | 10                                | 9            |                       |              |              |
| 0.88          | 10                                | 7            |                       |              |              |
| 0.44          | 10                                | 9            | 6.33 ± 2.51           | 9            | 6.33 ± 2.51 |
| 0.44          | 10                                | 6            |                       |              |              |
| 0.44          | 10                                | 4            |                       |              |              |
| 0.22          | 10                                | 2            | 3.33 ± 1.52           | 4            | 4.00 ± 1.00 |
| 0.22          | 10                                | 5            |                       |              |              |
| 0.22          | 10                                | 3            |                       |              |              |
| Control       | 10                                | 0            |                       | 0            | 0            |

# = Number of pests applied per petridish.
(Silica gel PF254) with solvent system ethyl acetate: cyclohexane (2 : 1) to afford compound 1. Using mass spectroscopy (HR/ES-MS), IR, ¹H-NMR, ¹³C-NMR, COSY, HSQC, HMBC, JMOD and NOESY the compound 1 was identified as a new triterpenoid α,3β,21β,23,28-penta hydroxyl 12-oleanene (Figure 3) [29,33].

Collection and maintenance of pest
The pest T. castaneum (Herbst) was originally collected from the Crop Protection Department of the University of Newcastle, U.K., and were maintained in the Crop Protection and Toxicology Laboratory of Department of Zoology, University of Rajshahi, Bangladesh. T. castaneum was reared in 1 L glass jar containing food medium, and a filter paper was placed inside each jar for easy movement of the pest. The jar was covered with a filter paper at the top, and kept in an incubator at 30 ± 0.5°C.

As food medium wheat flour and powdered brewers yeast in the ratio of 19 : 1 was used to culture the pest. Both flour and yeast were previously passed through a 250 micrometer aperture sieve and mixed thoroughly using an electric blender. The food was sterilized in an oven at 120°C for 6 h. Food was not used until at least 15 d after sterilization to allow its moisture content to equilibrate with that of environment.

Screening for insecticidal activity
Screening of insecticidal activity was carried out by surface film method [4,39,47]. The working solution was prepared by dissolving 100 mg experimental sample in 2 ml mixed solvent (50% chloroform + 50% methanol) in a vial. For each sample similar three vials were prepared.

Thirteen clean and dried petridishes (size of each is 60 mm, area of each is 28.26 cm²) were taken for each
sample. Four petridishes were marked by 50 mg, 25 mg, 12.5 mg and 6.25 mg. One ml working solution was poured into the 50 mg petridish and agitated clockwise, anticlockwise, left to right and right to left to further confirm the uniform dispersion, then 1 ml solvent (50% chloroform + 50% methanol) was added to that vial from which 1 ml had been used and mixed uniformly. From this vial, 1 ml solution was poured into the 25 mg petridish and agitated similarly for uniform dispersion. Using this serial dilution technique, likewise sample was poured into 12.5 mg and 6.25 mg petridishes and agitated similarly for uniform dispersion. The above processes were continued two times further using two remaining vials of working solution and eight remaining petridishes. Then the layers of dispersed sample into the petridishes were air dried. Further, 1 ml solvent (50% chloroform + 50% methanol) was poured and dispersed into control petridish and air dried.

The insects were collected by sieving and ten insects were applied on each layer of dispersed sample into the petridish. This process is continued for each petridish. Then the numbers of dead insects were recorded after passing 24 and 48 h.

Insect repellency test
Filter paper disc method [4,16,17,39,48] was used to determine repellency property of the triterpenoid. The working solution was prepared by dissolving 60 mg experimental sample in 2 ml mixed solvent (50% chloroform + 50% methanol) in a vial. For each sample similar three vials were prepared.

Nine clean and dried petridishes (size of each is 90 mm) and Nine filter papers (size-90 mm) were taken for each sample. Three petridishes were marked by 30 mg, 15 mg and 7.5 mg. Three filter papers were taken for these three petridishes and each filter paper was cut (by scissors) into equal two parts through centre, where one part can be used as control part and other part can be used as treated part. For 30 mg petridish with its filter paper, treated part of filter paper was taken at outer background of the petridish and 1 ml working solution (prepared previously) was dispersed uniformly thorough out this part of filter paper and air dried. Then this part of filter paper was joined with its control part using transparent adhesive tape and placed into the 30 mg petridish using forceps. Four petridishes were marked by 50 mg, 25 mg, 12.5 mg and 6.25 mg. One ml working solution was poured into the 50 mg petridish and agitated clockwise, anticlockwise, left to right and right to left to further confirm the uniform dispersion, then 1 ml solvent (50% chloroform + 50% methanol) was added to that vial from which 1 ml had been used and mixed uniformly. From this vial, 1 ml solution was poured into the 25 mg petridish and agitated similarly for uniform dispersion. Using this serial dilution technique, likewise sample was poured into 12.5 mg and 6.25 mg petridishes and agitated similarly for uniform dispersion. The above processes were continued two times further using two remaining vials of working solution and six remaining petridishes and filter papers.

Ten insects were applied at the center of each filter paper that present in the petridish. This process was continued for each petridish. Then the number of insects that repelled were counted per hour interval up to 5 h. The percentages of repellency were determined and results were analyzed through ANOVA after transforming them into arcsin percentage value.

Statistical analysis
The mortality data were subjected to probit analyses using SPSS (2001) to estimate LD50 of the compound against the stored product insect T. castaneum. The repellency data were calculated for percent repellency, which was again transformed using arcsine transformation for the calculation of analysis of variances (ANOVA). Mean values were compared using one way ANOVA (two factors without replication) (Additional file 1: Table S1).

Additional file

**Additional file 1: Table S1.** Two factors ANOVA (without replication) for repellency records through Arcsin transformation data.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
AK was responsible for conducting the experiments and manuscript preparation. MSI and MR supported AK in biological activities investigation, whereas TZ analyzed the data of biological activities. MEH supervised phytochemical works of the manuscript. All authors have read and approved the final manuscript.

Acknowledgements
The authors wish to thank Professor A.T.M. Naderuzzaman, Department of Botany, University of Rajshahi, Bangladesh for identification of the plant, and the Chairman, Department of Pharmacy, University of Rajshahi, Bangladesh for funding of the research.

Author details
1. Department of Pharmacy, University of Rajshahi, Rajshahi 6205, Bangladesh.
2. Department of Animal Husbandry and Veterinary Science, University of Rajshahi, Rajshahi 6205, Bangladesh.
3. Department of Fisheries, University of Rajshahi, Rajshahi 6205, Bangladesh.

Received: 5 October 2014 Accepted: 6 December 2014
Published: 15 December 2014

References
1. Smith JLW, Pratt JJJ, Nii I, Umina AP: Baking and taste properties of bread made from hard wheat flour infested with species Tribolium, Tenebrio, Trogoderma and Oryzaephilus. J Stored Prod Res 1971, 7:307–316.
2. Irshad M, Talpur S: Interaction among three coexisting species of stored grain insect pests. Pak J Zool 1993, 25:131–133.
3. Suresh S, White NDG: Mortality resulting from interactions between the red flour beetle and the rusty grain beetle. Proc Enomol Soc Mb 2001, 57:1–18.
4. Iram N, Anshad M, Akhter N: Evaluation of botanical and synthetic insecticide for the control of Tribolium castaneum (Herbst) (Coleoptera: Tenebrionidae). Bio Assay 2013, 8:1–10.

5. Abbasipour H, Mahmoudv M, Rasteeg F, Hosseinpour MH: Bioactivities of jimsonweed extract, Datura stramonium L. (Solanaeae), against Tribolium castaneum (Coleoptera: Tenebrionidae). Turk J Agric For 2011, 35:623–629.

6. Isman MB: Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. Annu Rev Entomol 2006, 51:45–66.

7. Dyte CF, Blackman DC: Laboratory evaluation of organophosphorous insecticides against susceptible and malathion-resistant strains of Tribolium castaneum (Herbst) (Coleoptera: Tenebrionidae). J Stored Prod Res 1972, 8:103–109.

8. Blunt JW, Copp BR, Munro K, Northcot PE, Prinsen MR: Marine natural products. Nat Prod Rep 2005, 22:215–61.

9. Papachristos DP, Starnopoulos DC: Toxicity of vapours of three essential oils to the immature stages of Acanthoscelides obtectus (Say) (Coleoptera: Bruchidae). J Stored Prod Res 2002, 38:365–373.

10. Umaotek SBA, Gerard MB: Comparative efficacy of Acorns calamus powder and two synthetic insecticides for control of three major insect pests of stored cereal grains. Glob J Agric Sci 2003, 2:94–97.

11. Zhang WX, Ling B, Chen SY, Liang GW, Pang XF: Repellent and oviposition deterrent activities of the essential oil from Mitkana micronatha and its compounds on Plutella xylostella. Insect Sci 2004, 11:37–45.

12. Tapondjou AL, Adler C, Fontem DA, Bouda H, Reichmuth C: Bioactivities of cymol and essential oils of Cupressus sempervirens and Eucalyptus saligna against Sphodopus zeamais Motschulsky and Tribolium confusum du val. J Stored Prod Res 2005, 41:91–102.

13. Ferrero AA, González JOW, Chopa CS: Biological activity of Schinus molle on Tribolium infestans. Fitoterapia 2006, 77:381–383.

14. Wang J, Zhu F, Zhou XM, Niu CY, Lei CL: Repellent and fumigant activity of essential oils from Artemisia vulgaris to Tribolium castaneum (Herbst) (Coleoptera: Tenebrionidae). J Stored Prod Res 2006, 42:339–347.

15. Rajendran S, Sriranjini V: Effect of some Ghanaian plant components on control of two Sitophilus zeamais grain insect pests in farmer stores and public ware houses. World J Agric Sci 2011, 7:527–540.

16. Asyemw MH: The potential of secondary metabolites in plant material as deterrents against insect pests: a review. Afr J Pure Appl Chem 2010, 4:243–246.

17. Long Z, Hock S, Hung S: Screening of Chinese medicinal herbs for bioactivity against Sitophilus zeamais Motschulsky and Tribolium castaneum (Herbst). J Stored Prod Res 2006, 43:290–296.

18. Moharramipour S, Raffa JN, Moravati M, Talea IJ, Fathipour Y: Effect of extract of Nerium oleander, Lavandula offi cinalis, and Frenula assafoetida on nutritional indices of Tribolium castaneum adults. J Entomol Soc Iran 2002, 23:69–89.

19. Shahkarami J, Kamali K, Moharramipour S, Meshkatladad MH: Effect of three plant essential oils on bioactivities of Collosobruscus maculatus F. (Coleoptera: Bruchidae). Iran J Agric Sci 2004, 35:965–972.

20. Farhana K, Islam H, Enman EH, Islam N: Toxicity and repellent activity of three spice materials on Tribolium castaneum (herbst) adults. J Bio Sci 2006, 14:127–130.

21. Luo D, Zhang X, Tian X, Liu J: Insecticidal Compounds from Tripterygium willfordii Active against Mythimna separata. Zeit für Naturforsch 2004, 59:421–426.

22. Pungitote CR, Garcia M, Gianello JC, Tonon CE, Sosa ME: Lethal and sublethal effects of triterpenes from Junelina aspera (Verbenaceae) on the grain storage insect Tribolium castaneum (Coleoptera: Tenebrionidae). Rev Soc Entomol Argent 2007, 64:45–51.

23. Isman MB: Plant essential oils for pest and disease management. Crop Prot 2000, 19:603–608.

24. Rozman V, Kalinovic I, Korunic Z: Toxicity of natural occurring compounds of Lamiaceae and Lauraceae to three stored product insects. J Stored Prod Res 2007, 43:349–355.

25. Pandey N, Brave D: Antioxidant activity of ethanolic extract of Annona squamosa Linn Bark. Int J Green Pharm Sci 2011, 2:1692–1697.

26. Jeffery GH, Bassett J, Mendum J, Denney RC: Vogel's Textbook of Quantitative Chemical Analysis, 5th edition. Harlow: England; Longman Group UK Ltd, 2000/161–162.

27. Khan A, Haque E, Rahman MM, Mohamidk A, Rahman M, Sultana N: A new triterpenoid from roots of Laportea crenulata and its antifungal activity. Nat Prod Res 2007, 21:959–966.

28. Shrivastava G, Rogers M, Wiselaki A, Panthee DR, Chen F: Plant volatiles-based insect pest management in organic farming. Crit Rev Plant Sci 2010, 29:123–133.

29. Khan A, Islam MS, Rahman M, Zaman T, Haque E, Rahman MM: Sub-acute toxicological studies 2a,3β,21β,23,28-penta hydroxyl 12-oleanene isolated from roots of Laportea crenulata Gaud. Asian Biomed 2011, 5:505–509.

30. Khan M, Haque E, Rahman MM: Antimicrobial and cytotoxic activities of Laportea crenulata. Fitoterapia 2006, 79:584–586.

31. Khan A, Haque E, Rahman MM, Mosaddik A, Rahman M, Sultana N: A new triterpenoid from roots of Laportea crenulata and its antifungal activity. Nat Prod Res 2007, 21:959–966.

32. Upadhyay RK, Ahmad S: Management strategies for control of stored grain insect pests in farmer stores and public ware houses. World J Agric Sci 2011, 7:527–540.

33. Khan A, Haque E, Rahman MM, Mosaddik A, Rahman M, Sultana N: A new triterpenoid from roots of Laportea crenulata and its antifungal activity. Nat Prod Res 2007, 21:959–966.

34. Upadhyay RK, Ahmad S: Management strategies for control of stored grain insect pests in farmer stores and public ware houses. World J Agric Sci 2011, 7:527–540.

35. Upadhyay RK, Ahmad S: Management strategies for control of stored grain insect pests in farmer stores and public ware houses. World J Agric Sci 2011, 7:527–540.