Introduction

Achatina fulica Bowdich (East African land snail or giant African land snail) and *Laevicaulis alte* Férussac (garden slug) in the family Achatinidae are major pests of a wide variety of agricultural crops and horticultural plants throughout the world (Raut & Barker, 2002). Snails and slugs severely damaged vegetable crops and affected rice cultivation in Orissa, India, in 1946–47. In Dhanbad, Jharkhand, India, molluscs spread widely and damage vegetable crops during the winter season. *Achatina fulica* is also a very common pest in tropical and subtropical regions. It may reduce crop yield by severely damaging tender leaves, stems, flowers, or fruits of vegetable and other cultivable crops. *Achatina fulica* also damages crops by acting as a vector for pathogens like *Phytophthora palmivora* Butler, *Phytophthora colocasiae* Rac., and *Phytophthora parasitica* Dastur. In addition, the decaying remains of *A. fulica* generates an offensive odor, and the high calcium carbonate content of the shells can alter soil properties.

Slugs have also been recognized as crop pests in recent years in many parts of the world (Barker, 2002; South, 1992). Slugs are major pests causing severe yield losses for winter wheat in different countries (Ester & Nijenstein, 1995; Barratt et al., 1994; Glen, 1989). The feeding pattern of *L. alte* on Indian crops, such as lettuce, spinach, and coriander, has also been reported (Raut & Panigrahi, 1990). Slugs dwell in the humid environment under cultivable crops, and they kill seedlings and damage the tender leaves and buds of young plants. They feed by scraping the surface of seeds, roots, stems, and leaves. Slugs damage cereals, oilseed rape, and many horticultural crops in the mild, damp conditions of northwest Europe (Port and Port, 1986). They damage the wheat fields by feeding on, hollowing out, and killing recently planted seeds. In corn and many small grains, slugs scrape strips in the leaves, leading first to window-pane damage and then to leaf shredding (Bolda, 2005).

Current chemical molluscicides sometimes provide inadequate control in horticultural crops (Port et al., 2003). So there is a demand for an improved and environmentally safe means of mollusc control. Common cultural practices for the eradication of molluscs pests include collection and killing by application of sodium chloride and application of molluscicides. But these chemicals are unacceptable due to their toxicity to the environment. Because of the high solubility and mobility of chloride in soil, continuous application of sodium chloride may lead to high soil salinity and alteration of the sodium adsorption ratio of cultivable land. Other chemicals used for slug control are Deadline M-Ps (metaldehyde), Deadline Bullets (metaldehyde), and Sluggo (iron phosphate). Recently, the use of food-grade caffeine for control of slugs has been reported (Bolda, 2005). Caffeine applied at a concentration of 1%–2% was tested in ornamental plant cultivation and was found to be a lethal neurotoxin for slugs. Current mollusc control relies largely on molluscicides based on metaldehyde and carbamate compounds, which are often ineffective and can affect non-target organisms (Bailey, 2002).

Silica from rice husk ash (RHA) is a potential alternative to synthetic molluscicides for the control of mollusc pests. As a natural biogenic product, silica has a physical mode of action and does not pose any environmental or health concerns, like synthetic pesticides. It contains no chemical insecticide or knock-down agents, has zero mammalian toxicity, and leaves no harmful residues. However, to avoid environmental problems and concerns for human health, silica can be coated with biopesticides. Neem (*Azadirachta indica* A. Juss), karanj (*Pongamia pinnata* (L.)), tobacco (*Nicotiana tabacum* (L.)) and *Calotropis* (*Calotropis procera* (L.)) were used as the botanical pesticides because they are cost effective, nontoxic, biodegradable, and eco-friendly (Sateesh, 1998). Components isolated from neem, such as the tetracyclic triterpenoids meliantetyraolenone and odoratone, have been already tested for pesticidal activity against *Anopheles stephensi* Liston (Siddiqui et al., 2003). The abundance of latex (containing alkaloids) in the green parts of *C. procera*, which belongs to the family Asclepiadaceae, may be used by the plants as a defense strategy against insects (Larhsini Metal, 1997). Alkaloids present in an ethanol extract of *C. procera* leaves were reported and tested against *Musca domestica* (L.) by Begum et al., 2010**.** *Pongamia glabra* (Vent.) has also been reported for control of green leafhopper and brown plant hopper Ramaraju and Babu, (1989).

In the present study, we aim to report the molluscicidal effects of pesticide-coated silica from RHA on *Achatina fulica* (giant African land snail) and *Laevicaulis alte* (garden slug)

Materials and methods

Adult snails (*A. fulica*) and slugs (*L. alte*) of similar size and weight were collected from a vegetable field in Patherdih, Dhanbad. The collected snails and slugs were kept in a controlled-environment chamber set at 25^{o}C and 70% relative humidity to become acclimatized to the experimental conditions.

Biogenic silica was obtained from RHA with the Kamath and Proctor (1988) method. An x-ray diffractometer (model D8 Advance, M/S Bruker AXS, Germany) was used for phase identification of the biogenic silica. A trinocular research microscope (model Eclipse 80i Nikon, Japan) and a scanning electron microscope (SEM) were used to observe the surface structures of the silica particles.

Fresh leaves of neem, karanj, tobacco, and *calotropis* were collected and properly cleaned from the institute's field in Dhanbad. Aqueous leaf extract was pulverized with double distilled water for the neem, karanj, and tobacco and with 90% ethanol for the *calotropis* (Begum et al., 2010). The extract was centrifuged at 3000 rpm for 10 min. Further, the extract (10 mL) was mixed with biogenic silica (1 g). The mixture was blended in a vortex mixer for 10–15 min. The blended biogenic silica and leaf extract slurry were air dried. The samples were stored in airtight containers at room temperature.

Dust and slurry forms of microsilica from RHA were applied on *A. fulica* and *L. alte*. Additional silica from RHA was coated with leaf extract from neem,

karanj, tobacco, and *calotropis*. The treatments—biogenic silica and botanical pesticides—were applied at the following concentrations: dust and slurry forms of silica, 0.05, 0.10, 0.15, 0.20, and 0.25 g; neem-coated silica, 0.20 g; karanj-coated silica, 0.20 g; *calotropis*-coated silica, 0.20 g; tobacco-coated silica, 0.20 g; uncoated silica, 0.20 g; and common salt, 0.20 g. One dose of each silica application and the control (untreated) were assessed, with three replicates of each treatment. Observations of the inactivation, mortality time, and percentage of liquid loss were recorded at 24 h intervals.

Results

Effects of silica on *Achatina fulica*: The silica extracted from RHA was white, with a particle size of 1–10 µm. X-ray diffraction analysis of this silica showed the predominant phase of quartz (SiO_2) in amorphous form. The SEM images showed that the silica particles had sharp edges (Fig. 1).

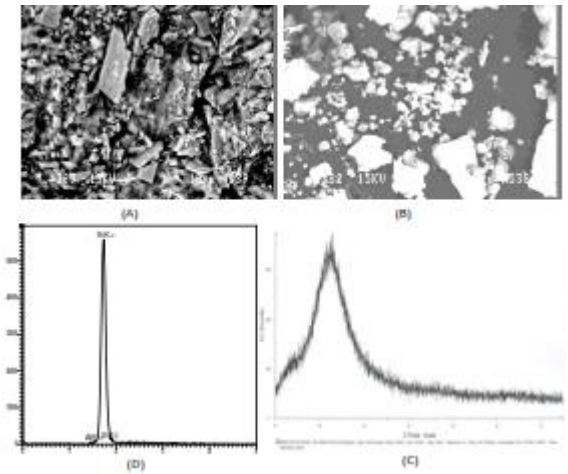

Fig. 1. (A) and (B) SEM image of silica extracted from RHA, (C) spectroplus result of silica from RHA, (D) XRD image of silica from RHA.

Among the different concentrations of dust and slurry forms of silica, the most rapid mortality for *A. fulica* was observed in the case of dry dust. After application of silica, the effects on the molluscs started within 3–5 s, and the inactivation stage was attained within 15–20 s at the highest concentration (0.25 g) of dust. The slurry form of silica also had similar effects at concentrations of 0.20–0.25 g (Fig. 2a). There was a considerable amount of liquid loss within a few minutes after treatment with the dust form of silica. The observed body fluid losses were 25%–42% for the dry dust and 17%–22% for the slurry application, at various concentrations of silica (Fig. 2b). During the experiment, it was observed that silica acted as a desiccant and led to total mortality of *A. fulica* within 26 min at a concentration of 0.25 g for both slurry and dust applications. The application of silica at lower concentrations (0.05–0.15 g) also led to the mortality of *A. fulica*, but it took a longer time (50–56 min) (Figs. 2c and 6a–d).

When the biopesticide-coated silica was applied on *A. fulica*, the snail started moving hyperactively, but within 3–5 min the movement slowed down and the snail became inactive. The coated silica was most effective when the biopesticide was neem, followed by tobacco and then karanj. The minimum time of inactivation, 17.5 s, was observed with tobacco-coated silica (Tsi) (Fig. 3a). The maximum amount of liquid loss occurred with uncoated silica (24.52%), followed by Tsi (24.45%) and neem-coated silica (Nsi) (23.18%) (Fig. 3b). The minimum time of mortality was observed with uncoated silica (71 min), followed by Nsi

(73.5 min) (Fig. 3c). The plant extract (liquid as well as dry powder) without silica showed no molluscicidal effect on *A. fulica*, except some temporary inactivation, particularly with tobacco extract. The application of sodium chloride, a common practice for farmers, also led to death, but the times in mortality were of long duration.

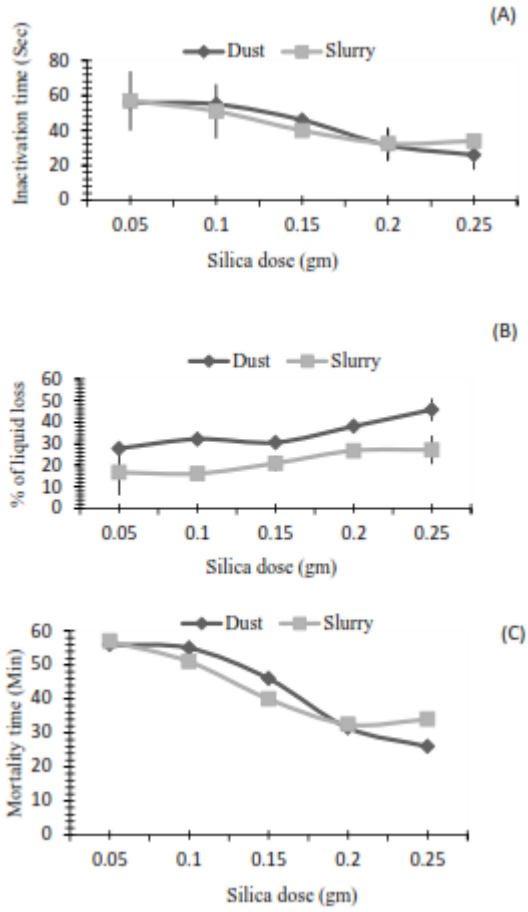

Fig. 2. (A), (B) and (C) Inactivation, liquid loss (%) and mortality rate of *A. fulica* on exposure to dry dust and slurry form of silica from RHA.

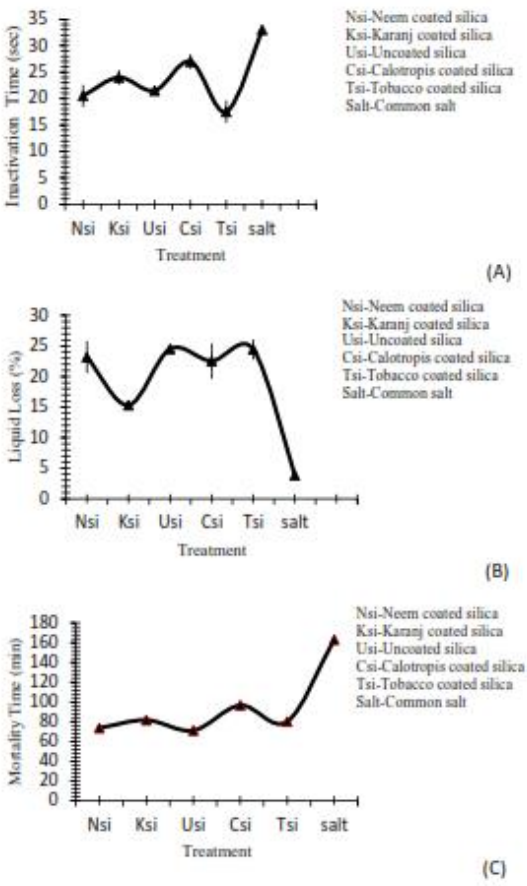

Fig. 3. (A), (B) and (C) Inactivation, liquid loss (%) and mortality rate of *A. fulica* on exposure to plant extract coated silica from RHA

Effects of silica on the slug *Laevicaulis alte*: It was observed that dry dust application of silica led to rapid mortality of the garden slug *L. alte*. The dust form of silica was much more effective than the slurry form. The effects on slugs started within 10–25 s of application of the dust, and the inactivation stage was reached in 3–10 min. The dust application of silica at 0.20–0.25 g per slug led to inactivation within 5 min, whereas it took 10 min when the silica was applied in a slurry (Figs. 4a and 7a–d).

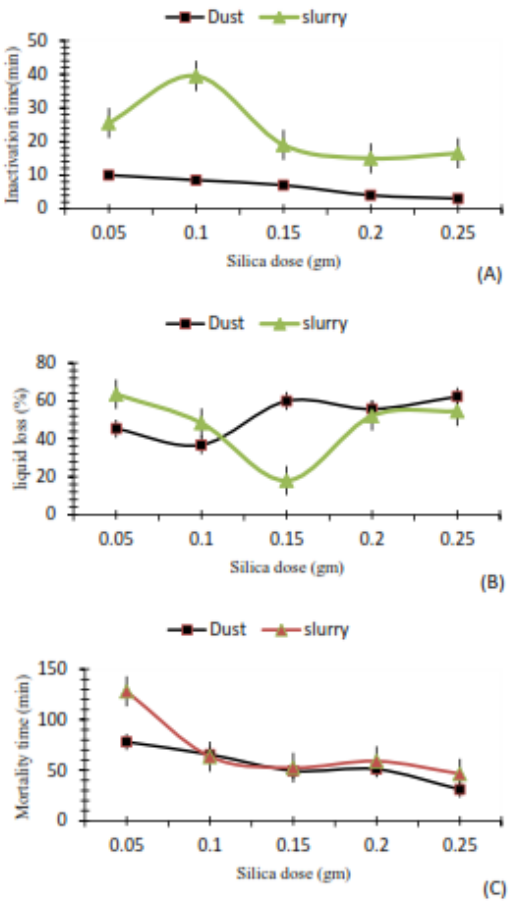

Fig. 4. (A), (B) and (C) Inactivation, liquid loss (%) and mortality rate of *Laevicaulis alte* on exposure to dry dust and slurry form of silica from RHA

Body fluid losses were higher (55%–67%) with dry silica dust applied at concentrations of 0.10–0.25 g per slug. The slurry form of silica also gave similar results (Fig. 4b). At higher concentrations of silica treatment (0.20–0.25 g), mortality was attained within 22–25 min (Fig. 4c). The minimum time of inactivation (4 min) was observed with *calotropis*-coated silica (Csi) (Fig. 5a), and the minimum time of mortality was with uncoated silica (18 min), followed by Tsi (31.5 min) (Fig. 5c). The maximum amount of liquid loss was shown with Tsi (49.51%), followed by Nsi (48.58%) and uncoated silica (48.51%) (Fig. 5b). The plant extract (liquid as well as dry powder) without silica showed no molluscicidal effect on the slugs.

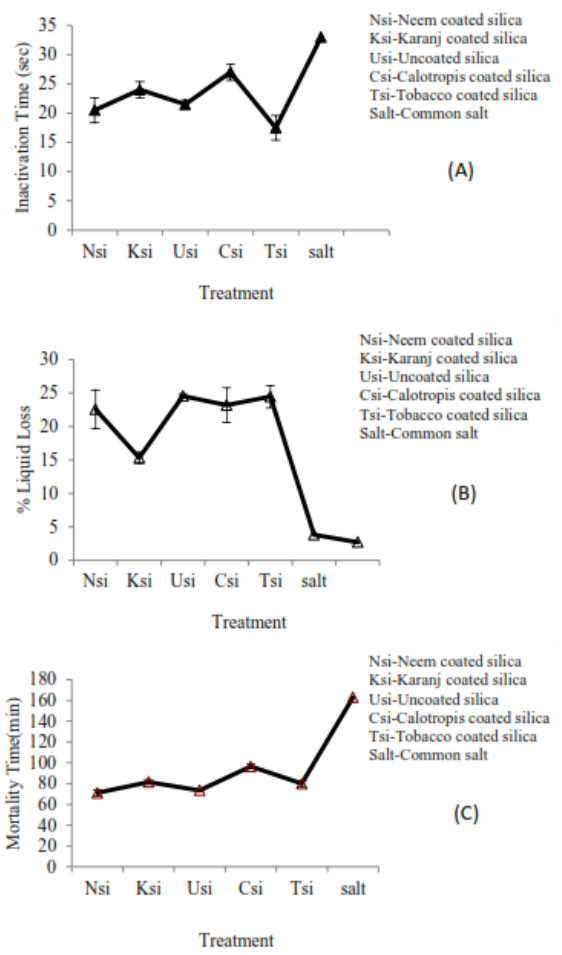

Fig. 5. (A), (B) and (C) Inactivation, Liquid loss (%) and mortality rate of *L. alte* on exposure to plant extract coated silica from RHA

Discussion

The present study revealed that biogenic silica in slurry form and in dust form, as well as in combination with other botanical pesticides, has a high molluscicidal effect on *A. fulica* and *L. alte*. During the experiment it was

observed that as the concentration increased (0.15, 0.20, and 0.25 g) the time of inactivation and mortality was reduced for *A. fulica* and *L. alte* with both slurry and dust treatments (Figs. 2, 4, 6, and 7). The silica particle became attached to the muscles of the snail, causing them to contract at regular intervals and resulting in the snail losing its body fluid. The time to reach inactivation and mortality depended on the weight of the mollusc, as well as the plant extract used to coat the silica. With neem-coated silica, mortality and inactivation were attained in less time. This may be due to the combined effects of silica and the pesticidal properties of the plant extracts. When silica became attached to the surface of the snail, the snail withdrew its tentacles and head into the shell, followed by complete retraction of the body, and started discharging body fluid continuously. After a few minutes, the discharge of body fluid stopped, followed by a loss in body weight. At this point, the snail was totally immobilized and considered dead.

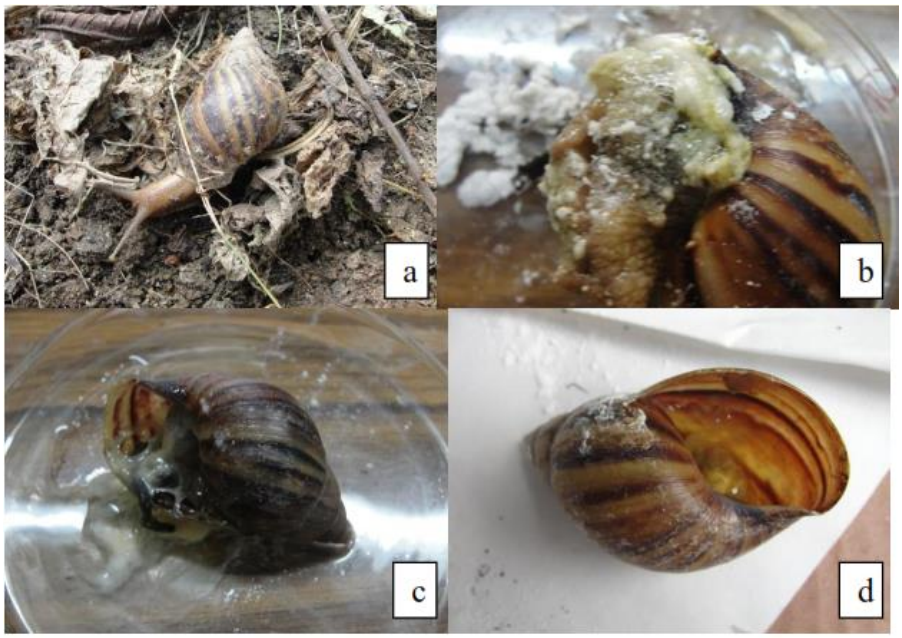

Fig 6. Effect of Silica (RHA) pesticides against *Achatina fulica*. a: control; b and c: treated and dehydration stage of *Achatina fulica*; d: total mortality of *Achatina fulica* after treatment.

Similar results were reported by Panigrahi and Rout (1998). In the present study, the dust application was more effective than the slurry, causing cuticular damage and high body fluid loss in both *A. fulica and L. alte*. The higher concentrations of silica (0.20–0.25 g per mollusc) were very effective, because the outer surface of the body was exposed to a greater number of microsilica particles, leading to quick inactivation and body fluid loss. Slurry applied at lower concentrations (0.05 and 0.10 g) did not have significant effects on the snails or slugs. This indicates that toxicity primarily depends on physical properties and not on chemical composition (Subramanyam and Roesli, 2000). Insecticidal mechanisms of silica may include

abrasion of the cuticle, absorption of cuticular waxes from the epicuticle surface, damage to the digestive tract, blockage of the spiracles and tracheae, and surface enlargement, combined with dehydration. The most widely accepted view is that damage to the cuticle is caused by removal or sorption of cuticular waxes, resulting in loss of water from the body and—depending on the relative humidity of the air—death through desiccation.

Fig 7. Effect of Silica (RHA) pesticides against *Laevicaulis alte.* a: control; b and c: treated and dehydration stage of *Laevicaulis alte*; d: total mortality of *Laevicaulis alte* after treatment.

Silica applications can significantly reduce damage from pests and diseases (Belanger et al. 1995; Ma and Takahashi, 2002; Meyer and Keeping, 2005). Current control methods rely largely on molluscicides of metaldehyde and carbamate compounds formulated as baited pellets, which are often ineffective and can also affect beneficial organisms (Bailey, 2002). Silica alone and in combination with biopesticides was very effective compared to conventional methods of controlling *A. fulica* and *Laevicaulis alte***.** The present research work showed that silica from rice husks can be used as a molluscicide to control *A. fulica* and *L. alte* without any environmental deterioration. Further investigation of changes in the hemolymph in silica-treated molluscs is currently ongoing in our laboratory.

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References

Bailey SER, 2002. Molluscicidal baits for control of terrestrial gastropods. Chapter 2 in Molluscs as Crops Pests, G.M. Barker ed., CAB International, Wallingford, UK.

Barratt BIP, Byers RA, Bierlein DL, 1994. Conservation tillage crop yields in relation to grey garden slug [Deroceras reticulatum (MUller)] (Mollusca: Agriolimacidae) density during establishment. Crop Protection **13**: 49-52.

Begum N, Sharma B, Pandey RS., 2010. Evaluation of Insecticidal Efficacy of Calotropis Procera and Annona Squamosa Ethanol Extracts Against *Musca Domestica*. Journal of Biofertilizers and Biopesticides **2010**: 1-1.

Belanger RB, Bowen PA, Ehret DL, Menzies JG, 1995. Solube silicon: its role in crop and disease management of greenhouse crops. Plant Disease **79**:329-336.

Bolda M, 2005. Slug control needed in strawberries UC Strawberry Farm Advisor Santa Cruz County. Calif. Western Farm Press, Nov. 19.

Ester A, Nijenstein JH, 1995. Control of the field slug Deroceras reticulatum (MUller) (Pulmonata: Limacidae) by pesticides applied to winter wheat seed. Crop Protection **14**: 409-413.

Glen D.M. (1989) Understanding and predicting slug problems in cereals. In: Slugs and Snails in World Agriculture (I.F. Henderson, ed.). British Crop Protection Council Monograph **41**: 253-262.

Kamath SR, Proctor A, 1988. Silica gel from rice hull ash, Arkansas University, USA.

Larhsini M; Bousad M; Lazrek HB; Jana M; Amarouch H. (1997) Evaluation of antifungal and molluscicidal properties of extracts of Calotropis procera. Fitoterapia **68**: 371-373.

Ma JF, Takahashi E, 2002. Soil,Fertilizer, and Plant Silicon Research in Japan.Elsevier Science Amsterdam, The Netherlands.

Meyer JH, Keeping MG, 2005. Impact of silicon in alleviating biotic stress in Sugarcane in South Africa. Sugarcane International **23**:14-18.

Port CM, Port GR, 1986. The biology and behaviour of Slugs in relation to crop damage and control. Agricultural Zoology Review **1**: 253-299.

Port GR, Collier RH, Symondson WOC, Bohan DA, Glen DM, 2003. Progress in improving the prediction and integrated control of slug damage in horticultural crops.In Slugs and Snails: Agricultural , Veterinary and Environmental Perspectives. pp. 301-306. Ed. GBJ Dussort. Alton UK: British Crop Protection Council Symposium, Proceedings No.80.

Ramraju K, Sundarbabu PC, 1989. Effect of plant derivatives on brown planthopper (BPH) and whitebacked planthopper (WBPH) nymphs emergence in rice. International Rice Research Newsletter **14**: 30.

Raut SK and Barker GM 2002. Achatina fulica Bowdich and other Achatinidae as pests in tropical agriculture. In: Barker GM (ed) Molluscs as crop pests. CABI Publishing, Wallingford, 55–114 pp.

Raut SK, Panigrahi A, 1990. Feeding rhythm in the garden slug Laevicaulis alte (Soleolifera: Veronicellidae). Malacological Review 23.

Sateesh MK, 1998. Microbiological investigations on die-back disease of neem (*Azadirachta indica* A.Juss.). Ph.D. thesis. University of Mysore. Mysore, India.

Siddiqui BS, Afshan F, Gulzar T, Sultana R, Naqvi SN, Tariq RM, 2003. Tetracyclic triterpenoids from the leaves of Azadirachta indica and their insecticidal activities. Chemical and Pharmaceutical Bulletin **51**: 415-417.

Singh RP, Raheja AK, 1996. Strategies in management of insect pests with neem (*Azadirachta indica* A.Juss.). In: Neem and Environment (Vol. I), (. Singh, R.P., Chari, M.S., Raheja, K., et al., eds.). Oxford and IBH publishing Co. Pvt. Ltd. New Delhi. India.

South A, 1992. Terrestrial Slugs: Biology, Ecology and Control. London: Chapman and Hall, 421 pp.

Subramanyam B, Roesli R, 2000. Inert dusts. In: Subramanyam, B & Hagstrum, D.W. (Eds.). Alternatives to Pesticides in Stored-Product IPM. Kluwer Academic Publishers. Dordrecht/Boston/London, 321–380 pp.