



Compatibility of Calneem oil and two parasitoids, *Habrobracon hebetor* (Hymenoptera: Braconidae) and *Venturia canescens* (Hymenoptera: Ichneumonidae) for protection of stored cereal grains against infestation by three stored-product insect pests

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- Red flour beetle, *Tribolium castaneum*
- *Corcyra cephalonica*
- *Cadra cautella*



- Microorganisms *
- Predators *
- Parasitoids *

Teretrius nigrescens



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Habrobracon hebetor

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Bacillus thuringiensis

Habrobracon hebetor (Braconidae)



- larval ecto-parasitoid
- 4 mm long
- 1000 wasps weigh 0.015 g (Schöller & Prozell, 1996)

Hosts → *Plodia interpunctella*, *Corcyra cephalonica*
Ephestia kuehniella, *Cadra cautella*
Ephestia elutella, *Sitotroga cerealella*

- Larval endoparasitoid
- size 6-10 mm
- develop. 25 D (25°C)



Hosts → *Plodia interpunctella*, *Ephestia kuehniella*
Ephestia elutella, *Galleria melonella*



Why compatibility of neem and biological control?



- **pesticide residues** – decreasing consumer tolerance
- **risks for environment** (methylbromide – destruction of ozone layer; phased out completely in 2005 in developed countries and by 2015 in developing countries)
- **pest resistance** to conventional pesticides



- no chemical residues
- no resistance
- targeted application
- continue in reproducing
- marginal risks for environment and human Health



- too high specialization
- slower effect
- more expensive



Aims and objectives of study

The overall goal of the research project was to enhance food security and safety as well as alleviate poverty in Ghana, by reducing losses in grains during storage with biological control using natural enemies and locally available botanicals as components of integrated control strategies.

- ◆ To assess the toxicity and protectant potential of CalO against the red flour beetle, *T. castaneum* in stored wheat
- ◆ To evaluate the compatibility of CalO and parasitic wasps, *H. hebetor* and *V. canescens* against *C. cephalonica* and *C. cautella* in stored rice as IPM
- ◆ To determine the effects of neem formulations on the reproduction and survival of *H. hebetor* and *V. canescens*

Questions

- ◆ Do different dosages of CalO significantly affect the emergence of the three stored product insect pests?
- ◆ Is CalO compatible with parasitic wasps, *H. hebetor* and *V. canescens*?



Bio-rational control of *Tribolium castaneum* in stored wheat with Calneem oil derived from neem seeds



Material & methods

Calneem™ oil



- CalO, (Calneem oil), a commercial biopesticide extracted from pure neem seed kernels by AQUA AGRIC Community Projects (AACCP) Ghana)
- CalO contains about 0.3% azadirachtin as its major active ingredient
- Calneem is marketed as 0.33% neem oil involving 50ml of neem oil + 10ml of emulsifier + 15 litres of water

Contact toxicity on filter paper

- Filter paper (90 cm diameter) placed in a glass Petri dish
- CalO (0.1-3.0% v/v) applied uniformly on the surface filter papers and air dried for abt 10 min
- 20 known age *T. castaneum* adults introduced separately into each dish
- Pirimiphos-methyl as reference insecticide and water as control
- treatment replicated five times and insect mortalities recorded after 24, 48 and 72 hours.

Contact toxicity topical

- Beetles picked individually and 1 μ m of each dose applied to the dorsal surface abdomen of each beetle using micro-pipette applicator
- After treatment, beetles transferred (20 insects/ Petri dish) containing food
- Insects examined daily 3 d and those that did not respond to three probings with a blunt probe were considered dead
- Insect mortalities were recorded at 24, 48 and 72 h

Persistence of CalO in grain Beetle mortality on grain

- Beetles were exposed to treated grain which has been stored for 1, 20, 30 and 60 d
 - 20 *T.c* adult introduced into the mixtures at appropriate d
 - Kept in the laboratory at $25\pm 1^{\circ}\text{C}$ and 65-70% rh.
 - Treatments replicated five times and mortality was recorded after 24 hours of exposure
- Test solutions were mixed with 500 g samples of wheat grain in 1-litre glass jars
 - Solvent allow to evaporate completely for 5 min
 - grains were infested with 20 *T. castaneum* adult
 - Treatment was replicated five times and mortality recorded 24, 48 and 72 hrs

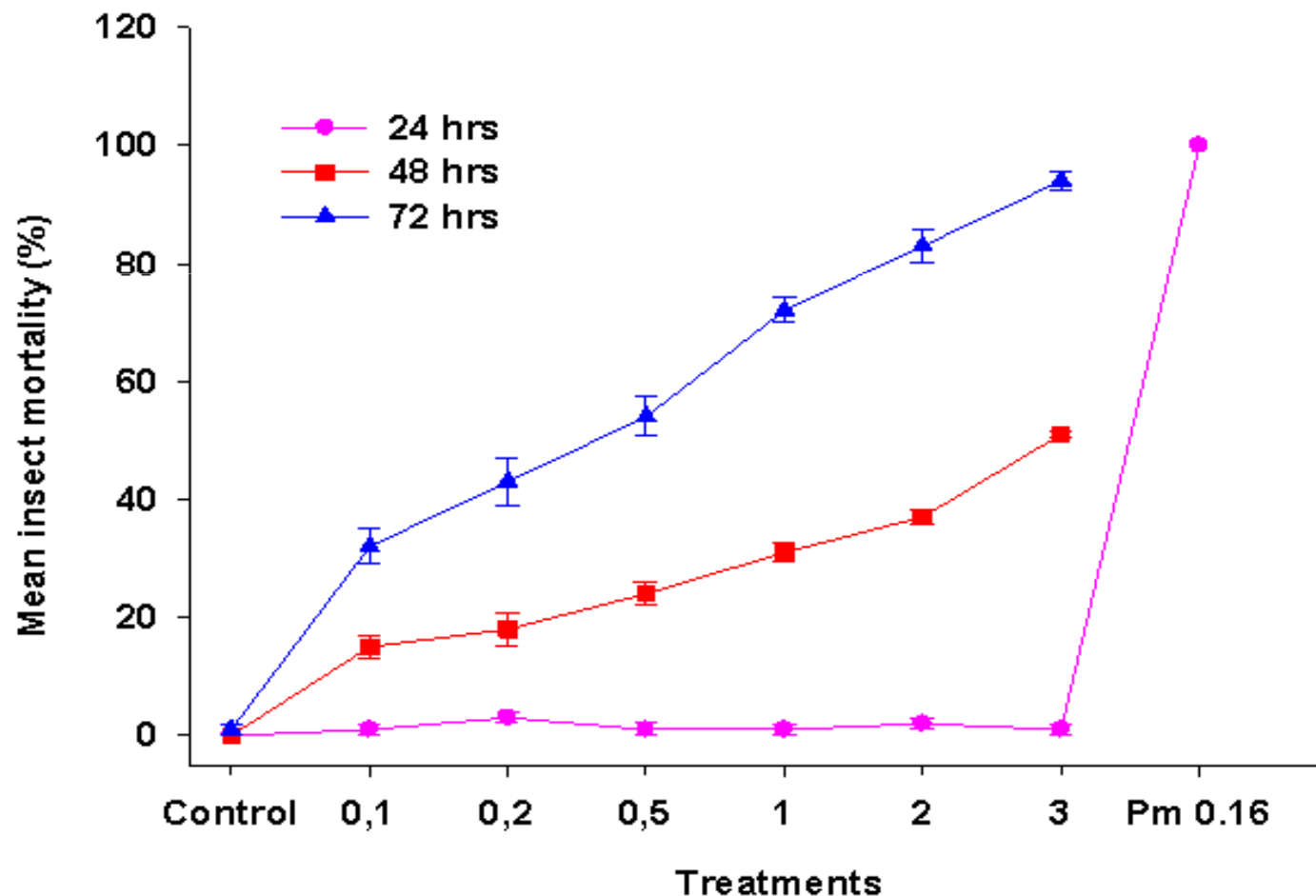
Toxicity of CalO to immature stages

- Batches of 100 g of wheat in 250 ml glass jars were infested with 20 unsexed known age *T.c* adults allow egg laying, parent adults removed after 1 week
- One day after adult removal, five batches of the grain were treated with different doses of CalO (0.1-3.0% v/v)
- Treatments repeated at one, two and three weeks after adult removal to determine effect on early instar larvae, late instar larvae and pupae, respectively
- Adults emerging jars counted for 8w following removal of adults

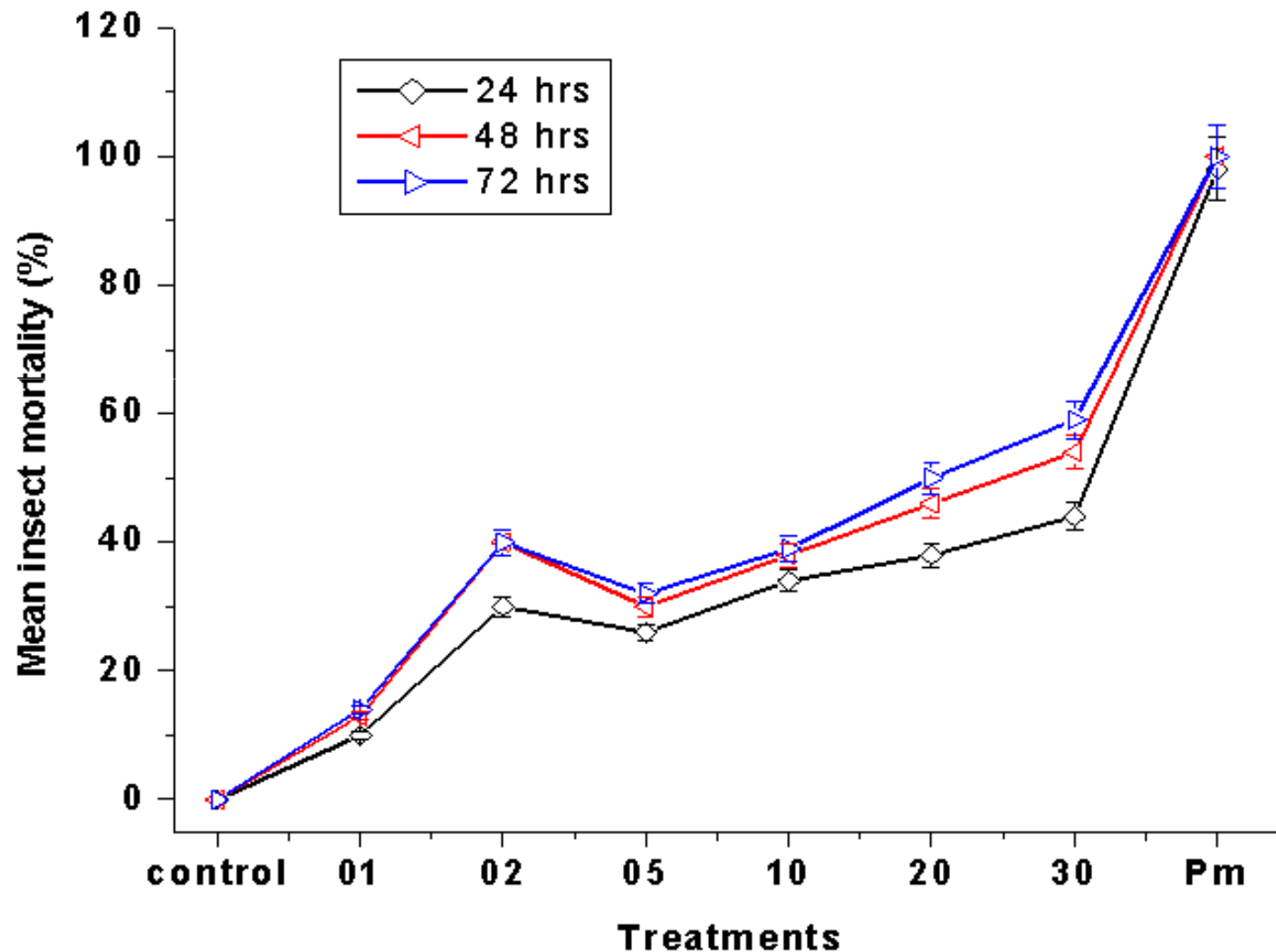
Repellency test

- Filter papers cut two equal half discs, treated dosages CalO aid of pipette, water applied other half control
- Both halves air dried abt 10 min then treated/untreated halves re-made together with sellotape
- The joined filter papers placed separate Petri dishes, 10 adult beetles released separately centre of each filter paper disc
- Numbers of insects present on control(N_c)/treated(N_t) after 24 h and computed Percent Repellency (PR) = $\{(N_c - N_t) / (N_c + N_t)\} \times 100$

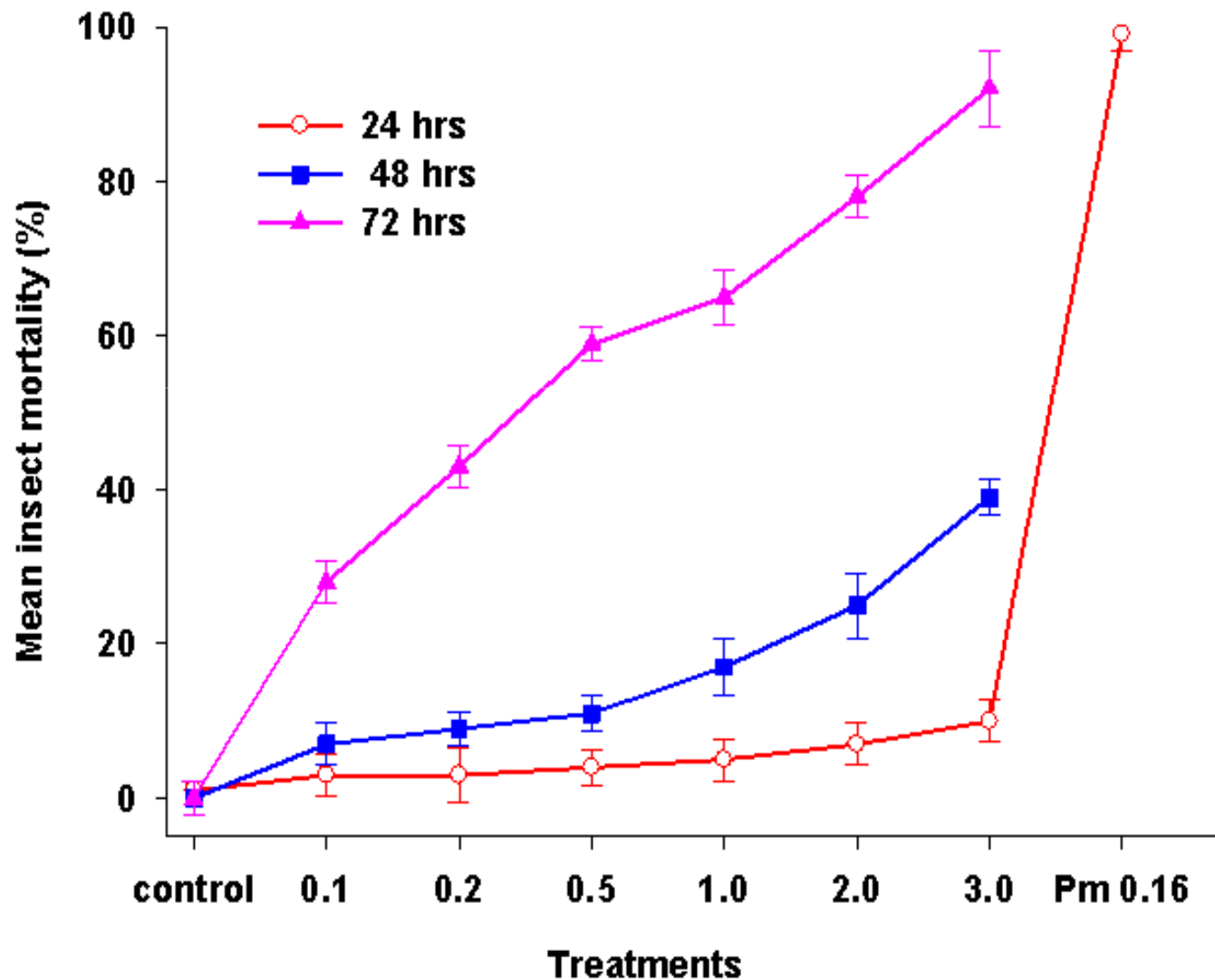
Toxicity of Calneem oil (0.1-3.0 % v/v) Pirimphos-methyl (0.16%) on filter paper to *T. castaneum*



Contact toxicity of different doses of CalO applied topically to the dorsal surface of *T. castaneum* using micro-pipette applicator

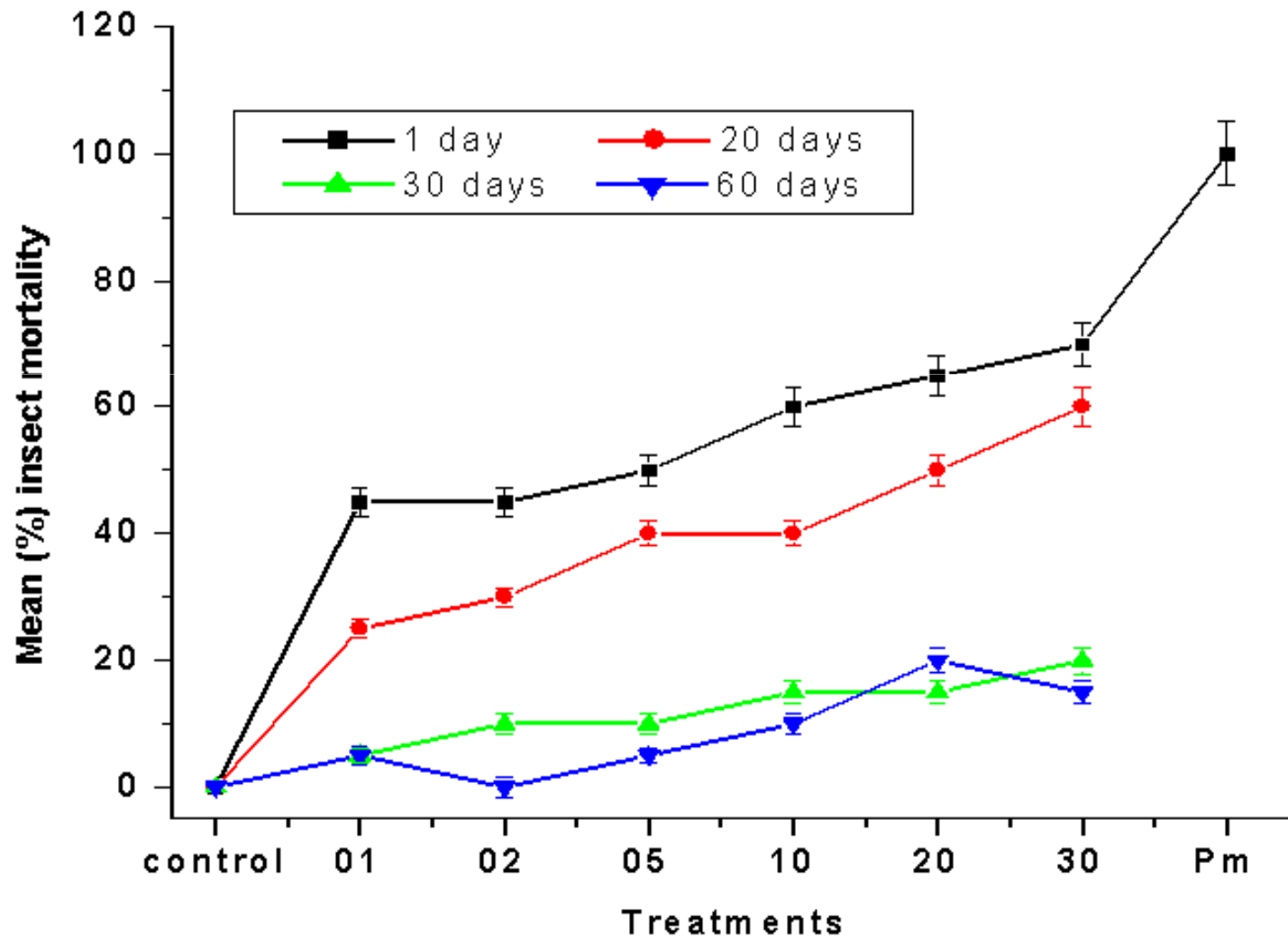


Toxicity of different doses of CalO and pirimiphos-methyl in wheat grains to *T. castaneum*



25±1°C, 65-70% rh, L16:D8

Mortality of *T. castaneum* in wheat exposed to different doses of CalO applied and stored for 1, 20, 30 and 60 days prior to exposure



25±1°C, 65-70% rh, L16:D8

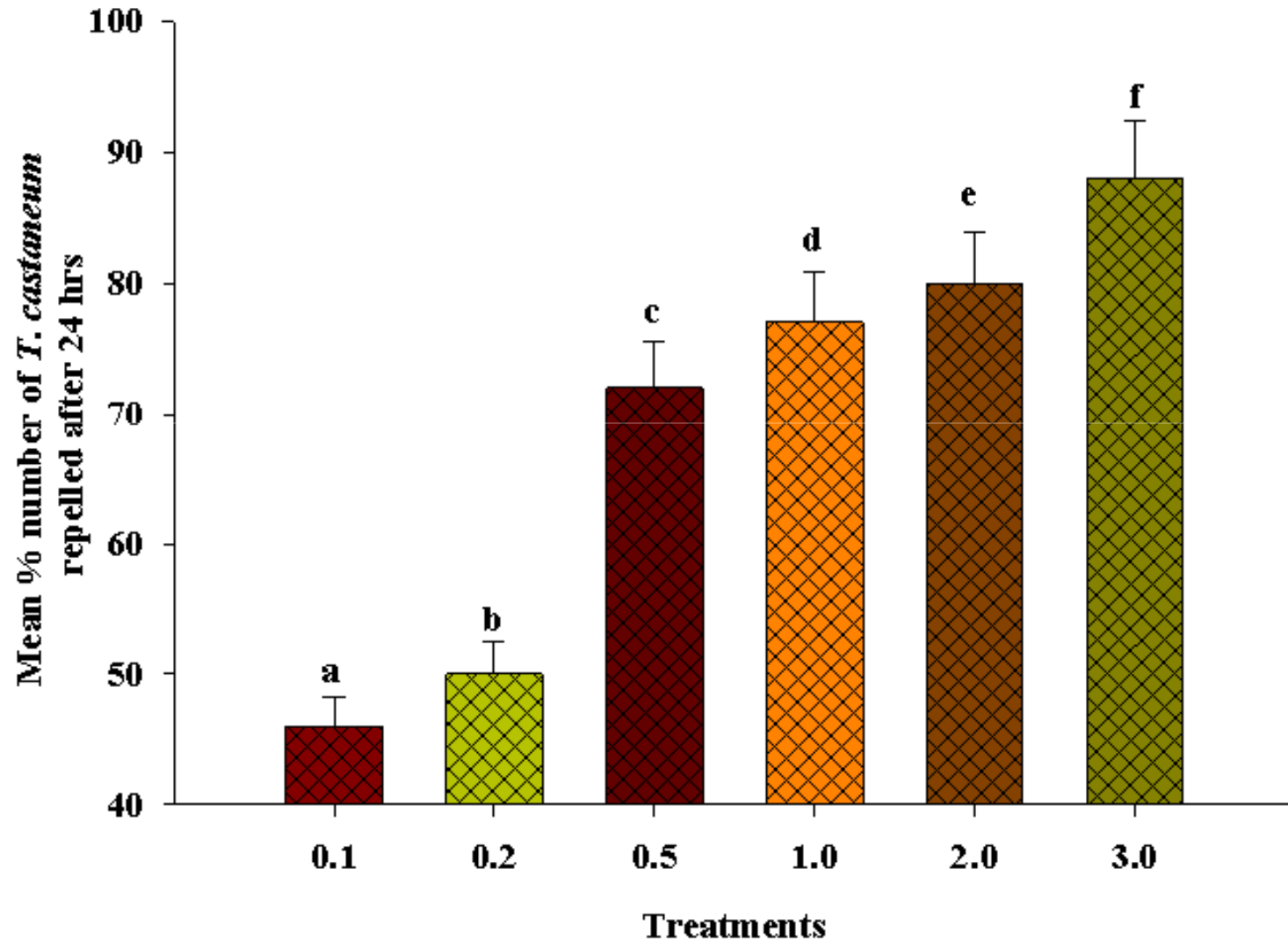
Mean number of *T. castaneum* adults that emerged from wheat treated with various dosages of CaO at different times after oviposition period

| Dosage V/V (%) | Eggs | Early larvae | Late larvae | Pupae |
|------------------|-------------|--------------|-------------|-------------|
| 0.0 | 308 | 312 | 309 | 305 |
| 1.0 | 81 | 83 | 78 | 85 |
| 2.0 | 45 | 50 | 50 | 55 |
| 3.0 | 20 | 22 | 26 | 23 |
| LSD at 5% | 14.0 | 12.0 | 12.2 | 12.5 |

25±1°C, 65-70% rh, L16:D8



Mean % repellency (PR) for different doses of CalO against *T. castaneum* adults in the choice arena



25±1°C, 65-70% rh, L16:D8

Bars denoted by different letter (s) are significantly different Student-Newman-Keuls Test ($P < 0.05$)

Material & methods



- 50 g of rice → *C. cephalonica*,
- 50 g of wheat → *C. cautella*
placed in 1 litre glass jars
- 20 last instar larvae of *C.c* & *C. cautella* were added in separate jars
- 10 freshly emerged, *H. hebetor* or *V. canescens* were introduced

Treatments comprised:

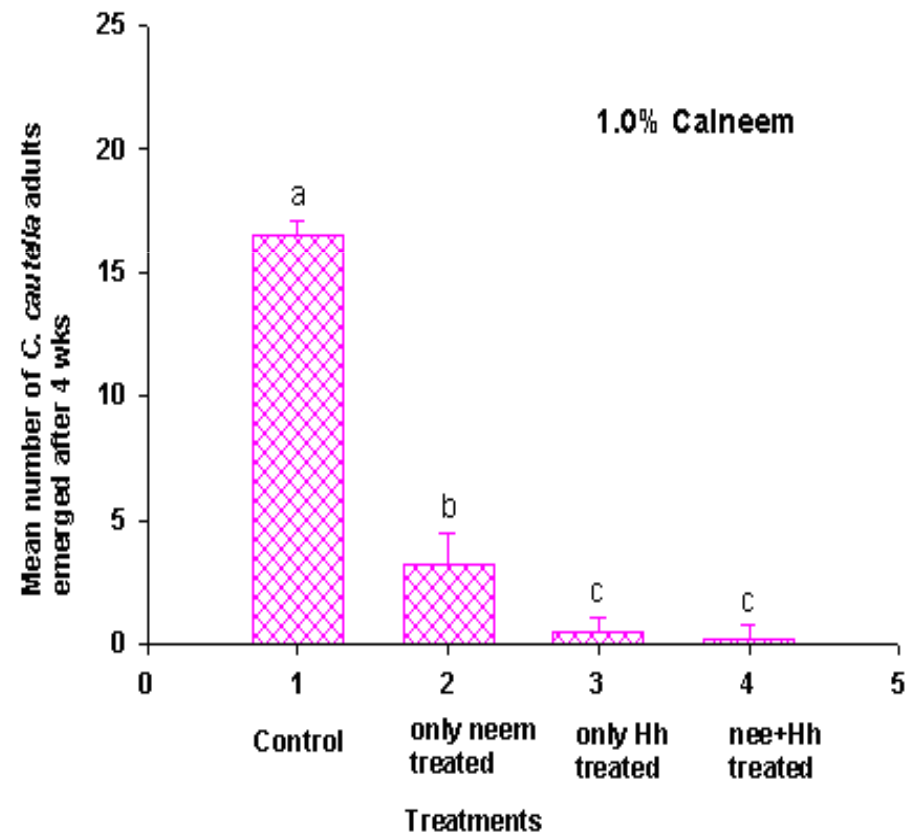
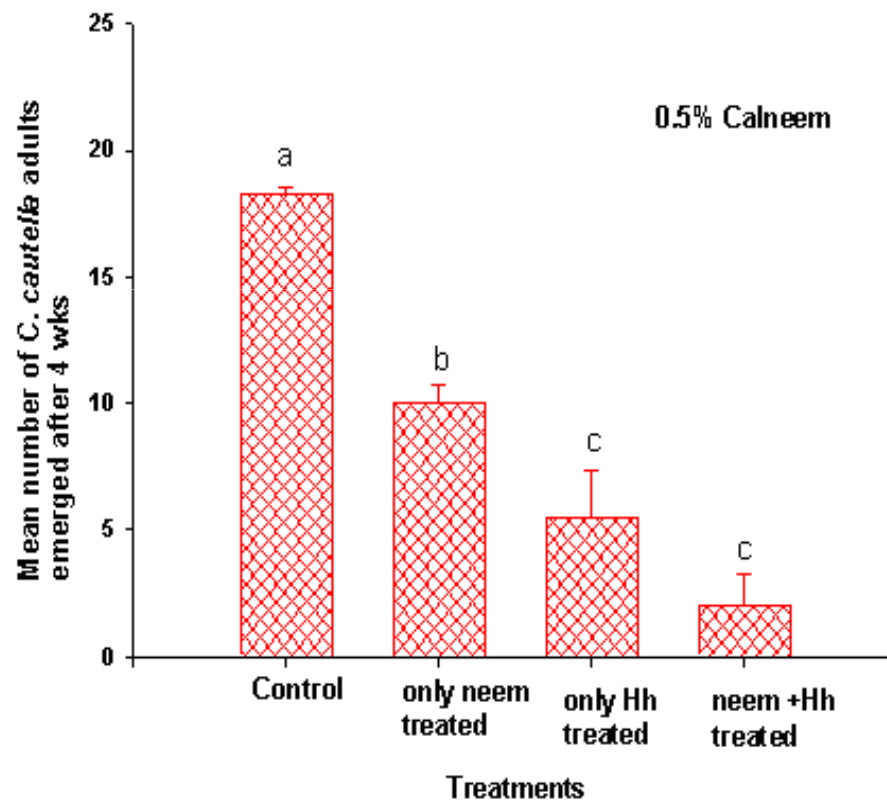
- grains treated with CalnO only,
- grain treated with 10 H.h and/or V.c. only,
- grain treated with CalnO +H.h. or V.c
- control with untreated grain
- grain treated with Neem+Hh or Vc

CalO dosages (0.5, -3.0%v/v)

Treatment replicated 4 times

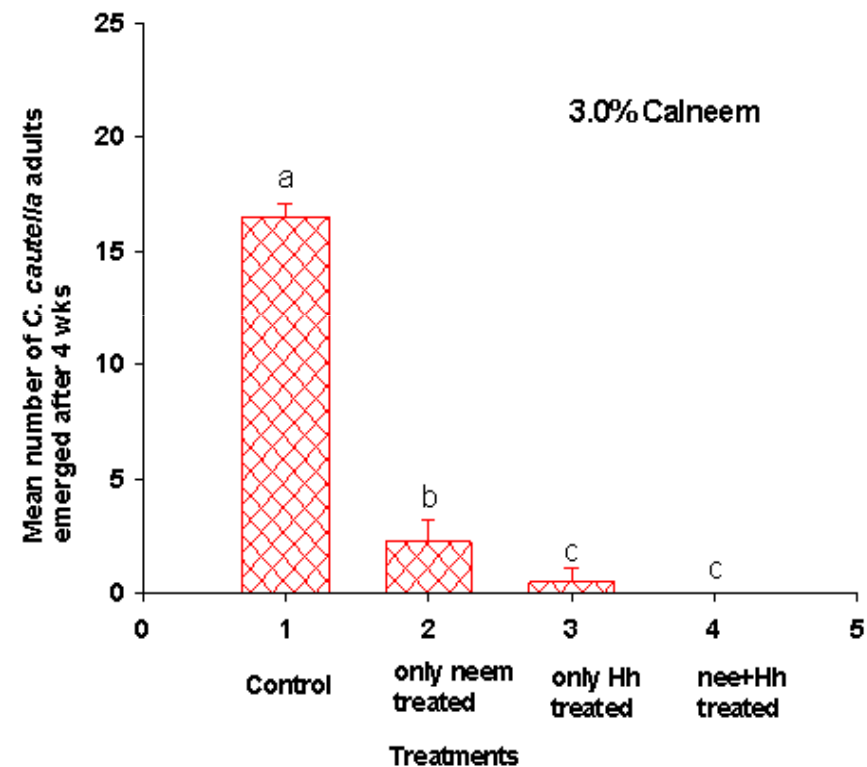
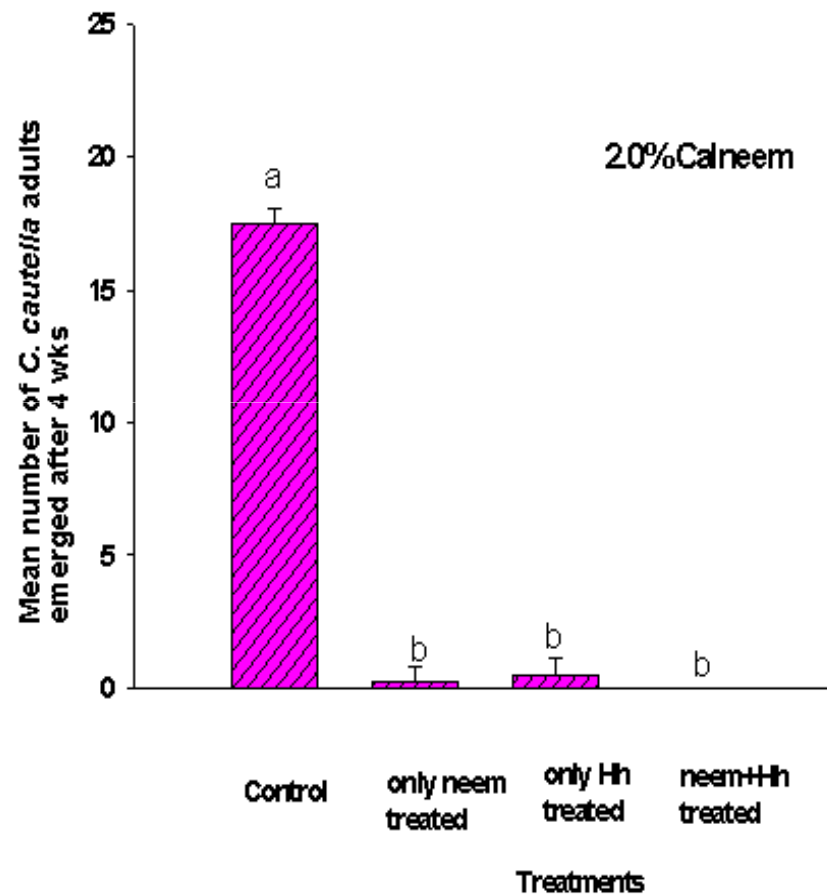
25°C & 65% r.h

Adult emergence of *C. cautella* in jars treated with CalO, *H. hebetor* alone or a combination of the two and the control



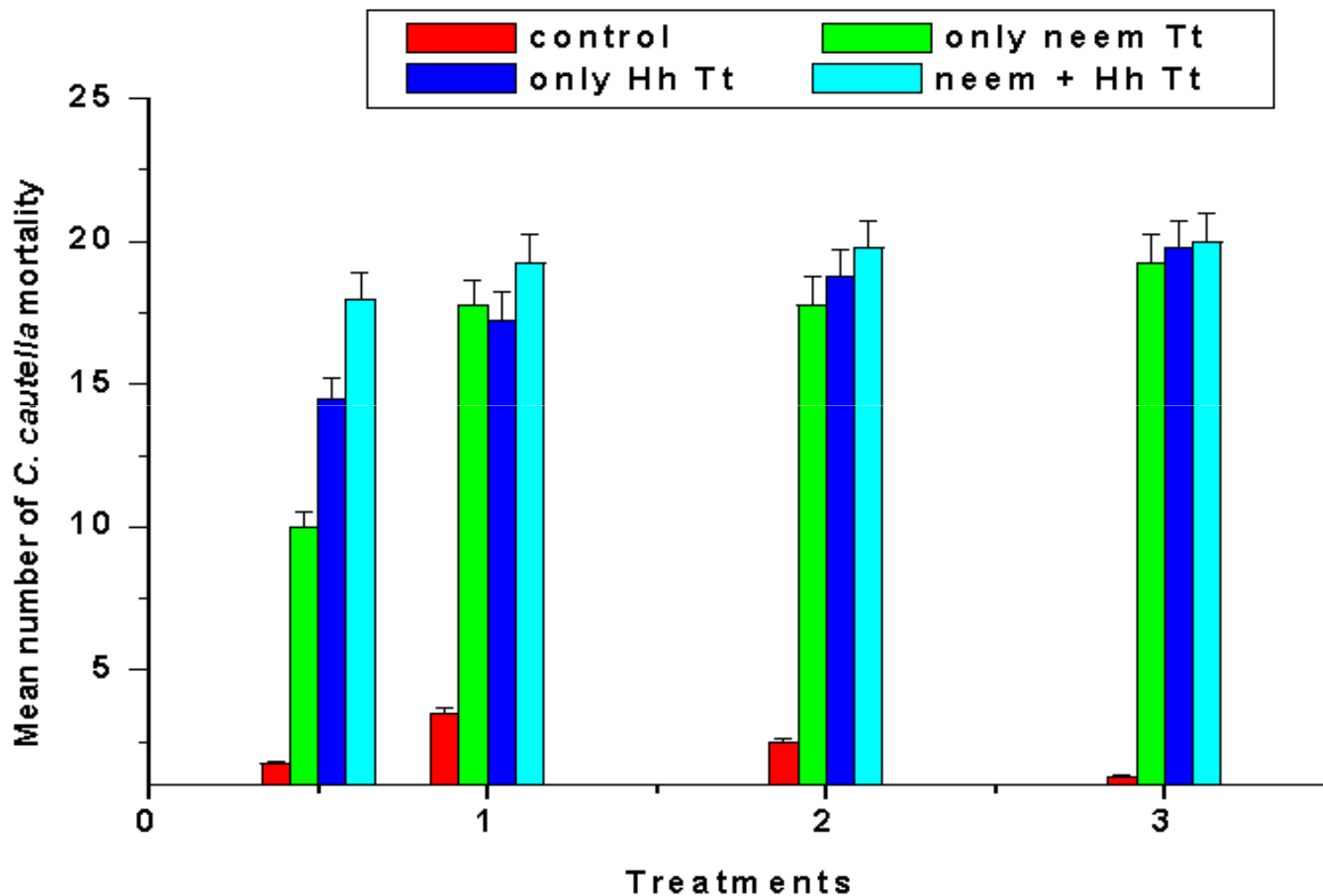
Bars denoted by different letter (s) are significantly different (Student Newman Keuls test, $P < 0.05$).

25°C & 65% r.h



Bars denoted by different letter (s) are significantly different (Student Newman Keuls test, $P < 0.05$).

Adult mortality of *C. cautella* in jars treated with CaO, *H. hebetor* alone or a combination of the two and the control



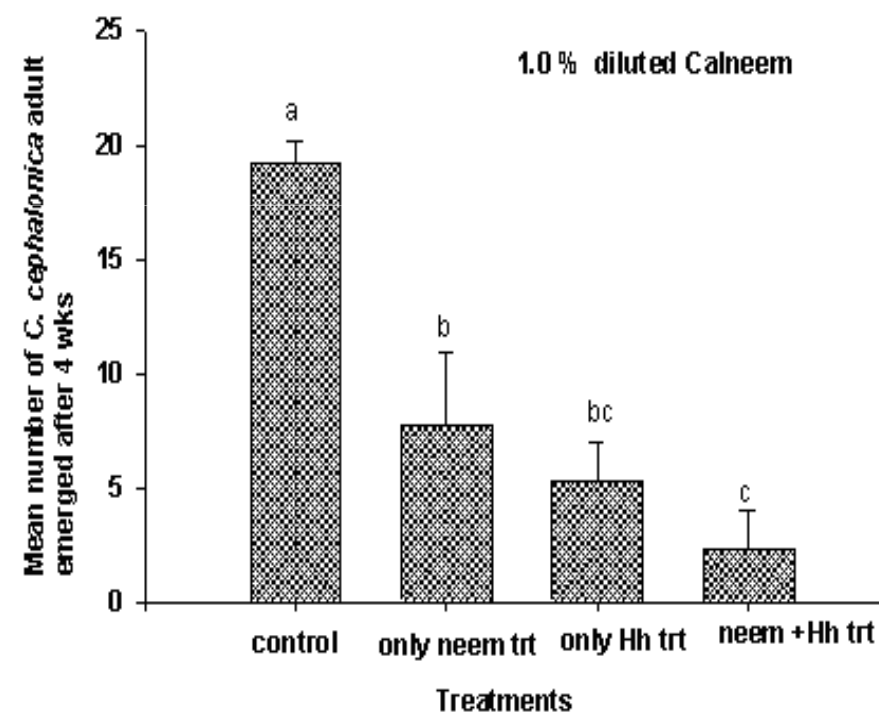
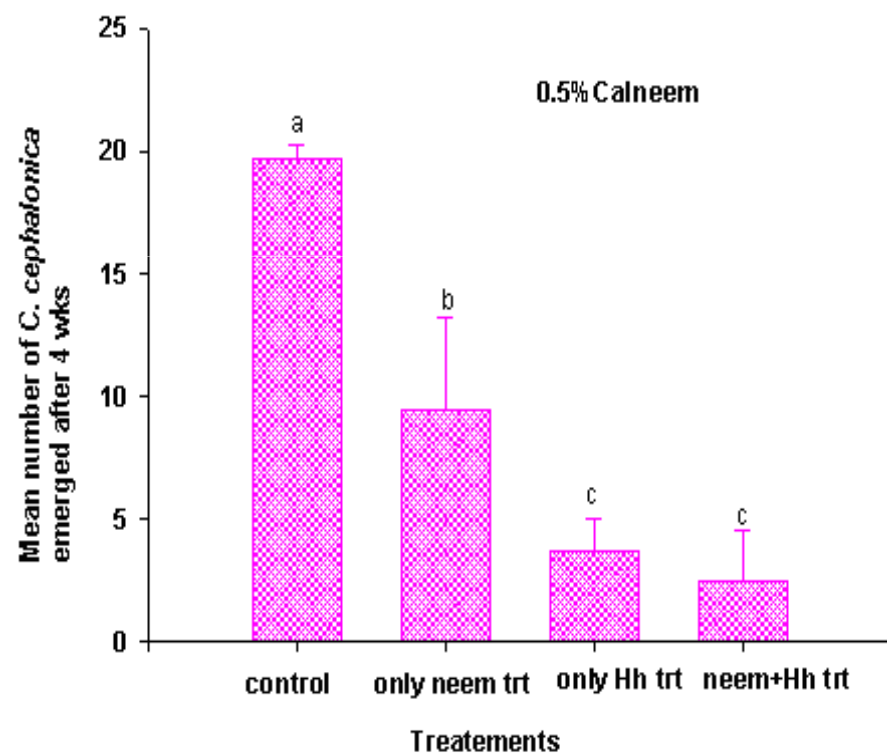
Mean of four replicates of 20 fourth instar larvae of *C. cautella*



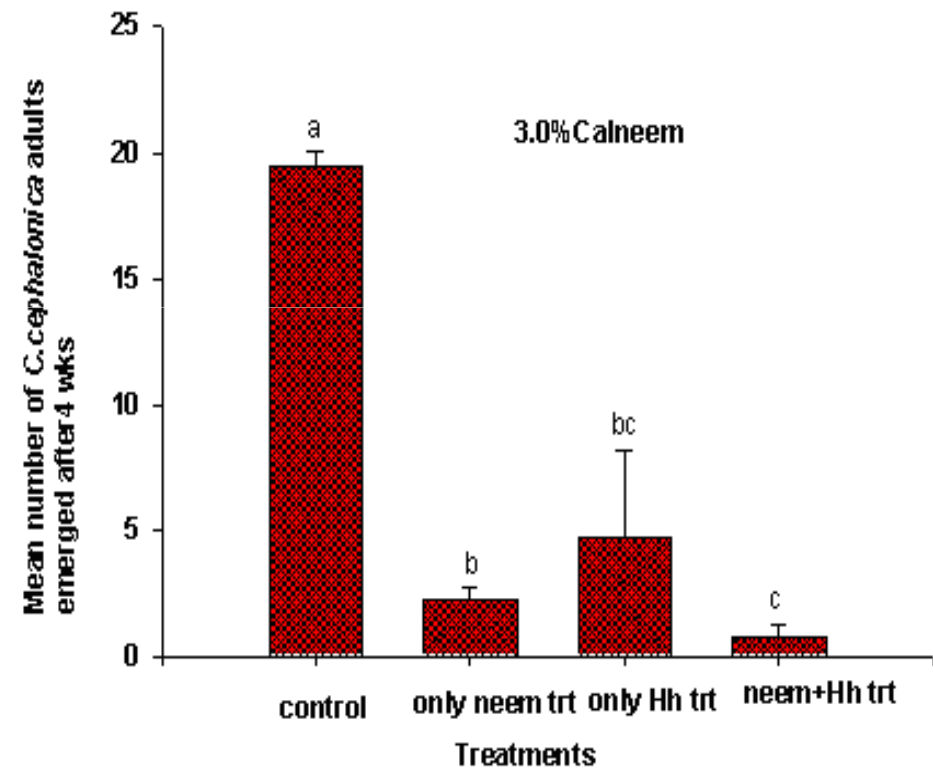
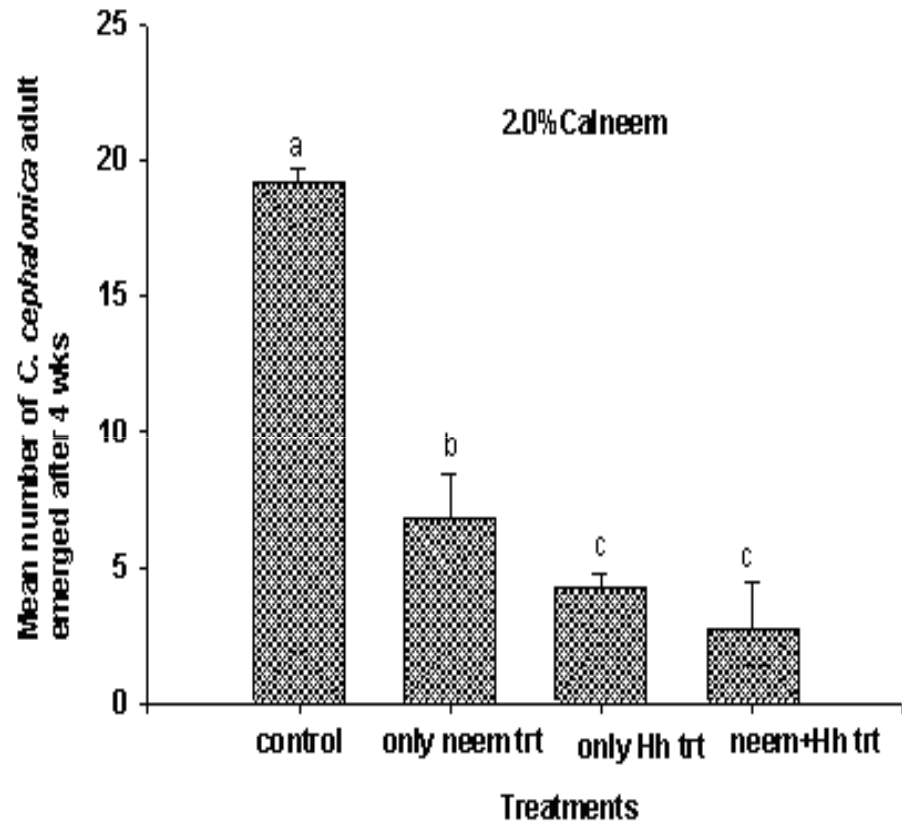
Adult emergence of *C. cephalonica* in jars treated with CalO, *H. hebetor* alone or a combination of the two and the control



25°C & 65% r.h



Bars denoted by different letter (s) are significantly different (Student Newman Keuls test, $P < 0.05$).

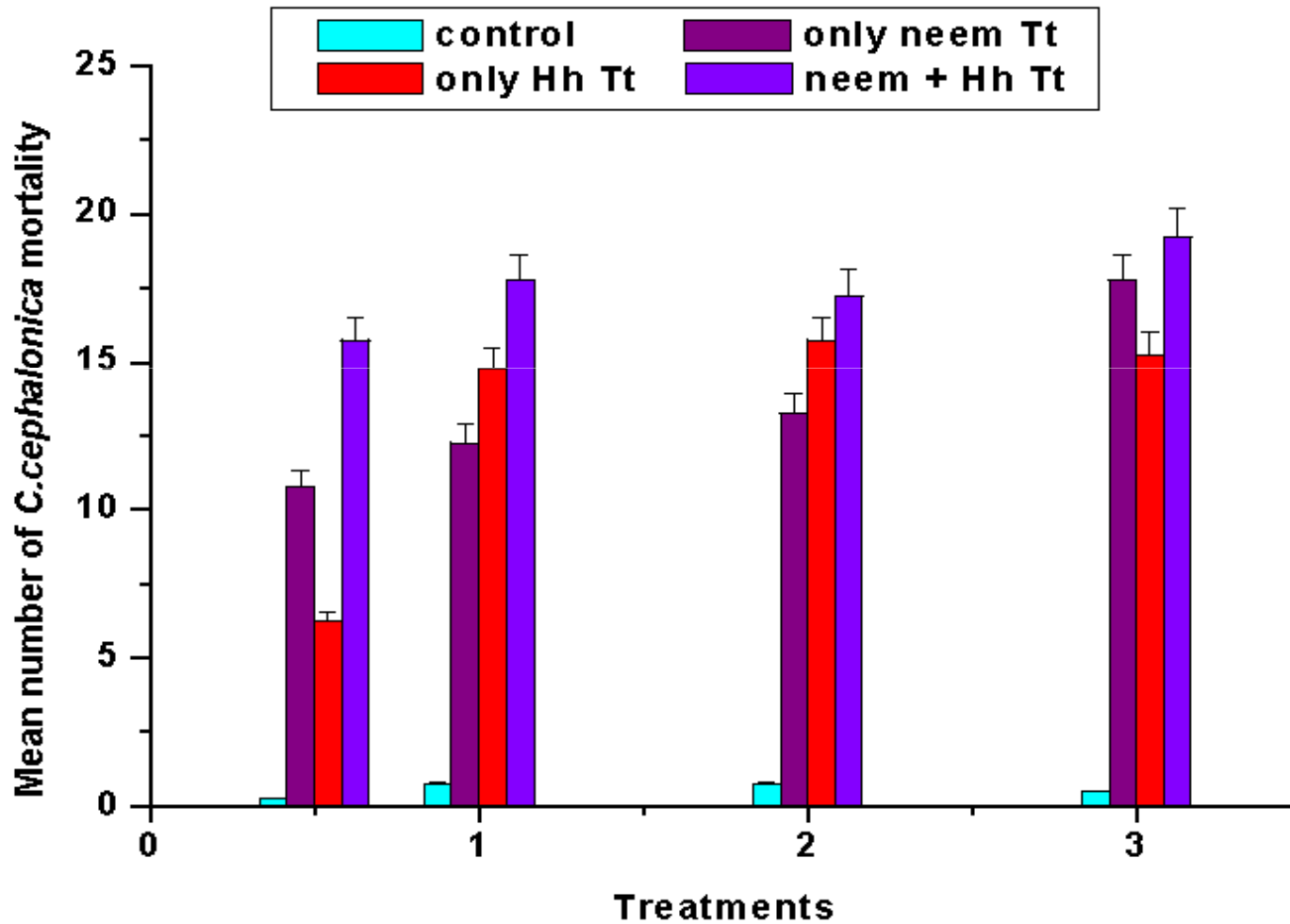


25°C & 65% r.h

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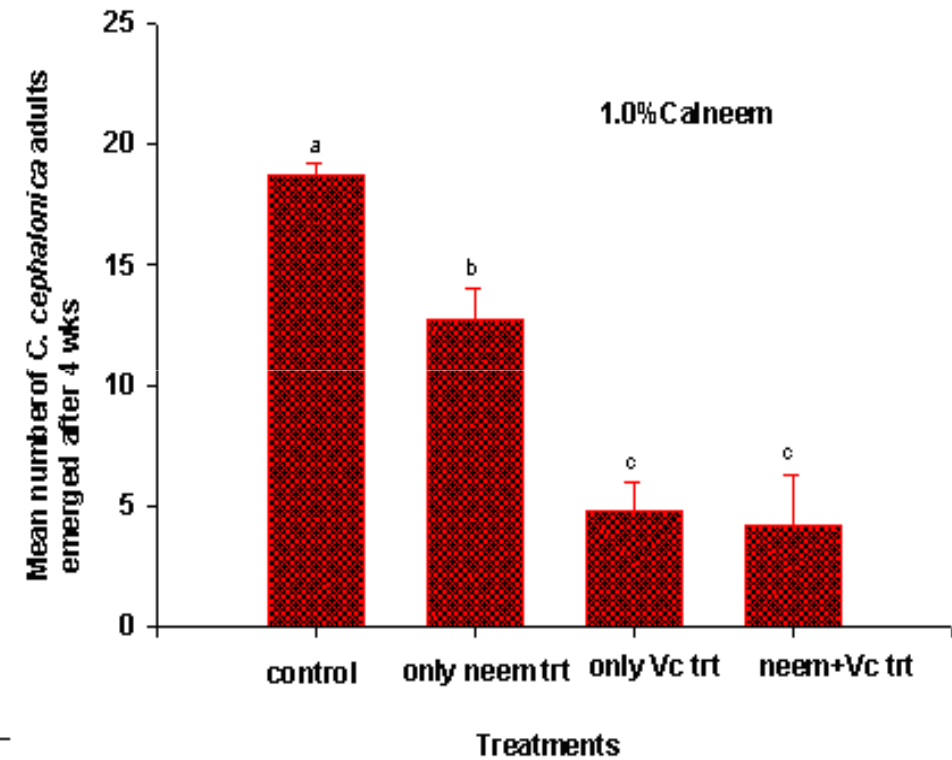
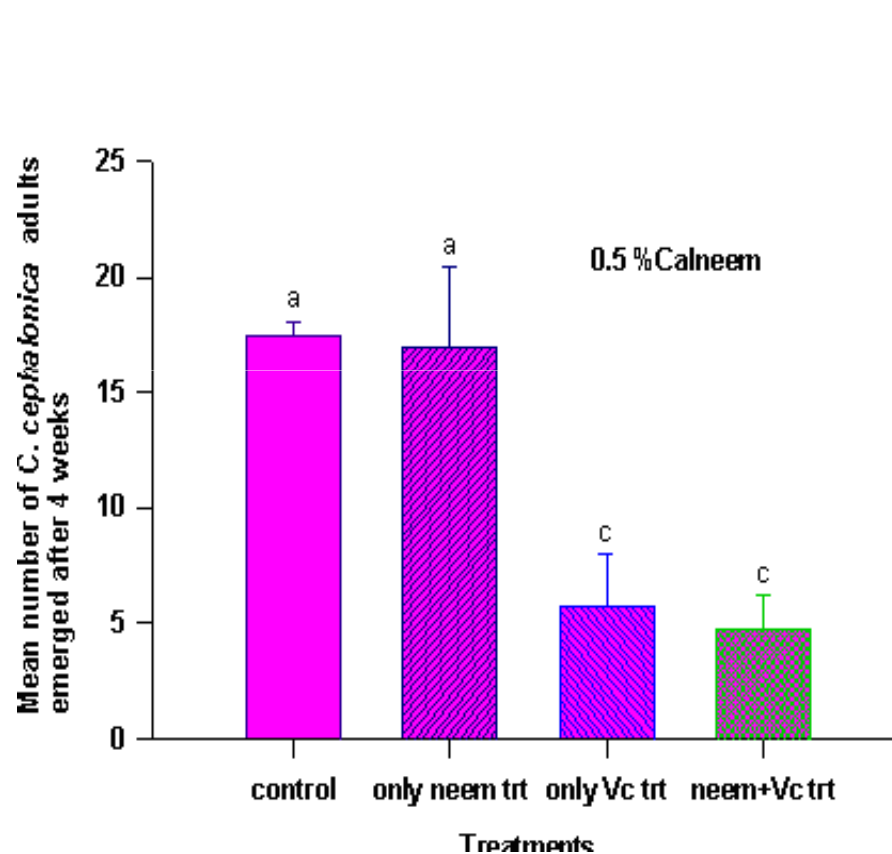
Adult mortality of *C. cephalonica* in jars treated with CalO, *H. hebetor* alone or a combination of the two and the control



Mean of four replicates of 20 fourth instar larvae of *C. cephalonica*

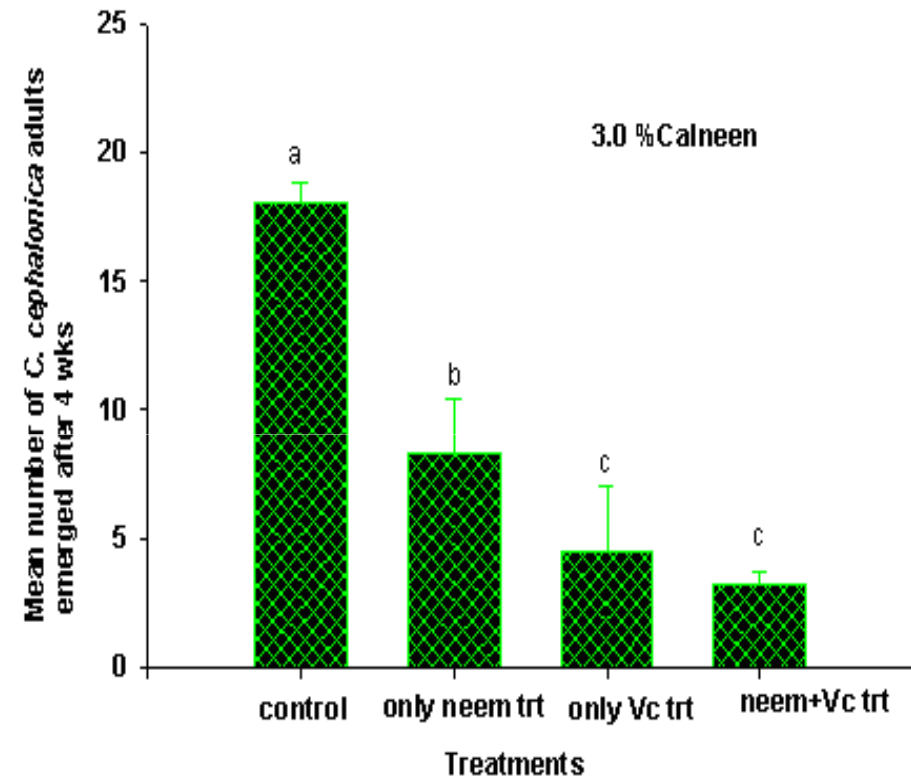
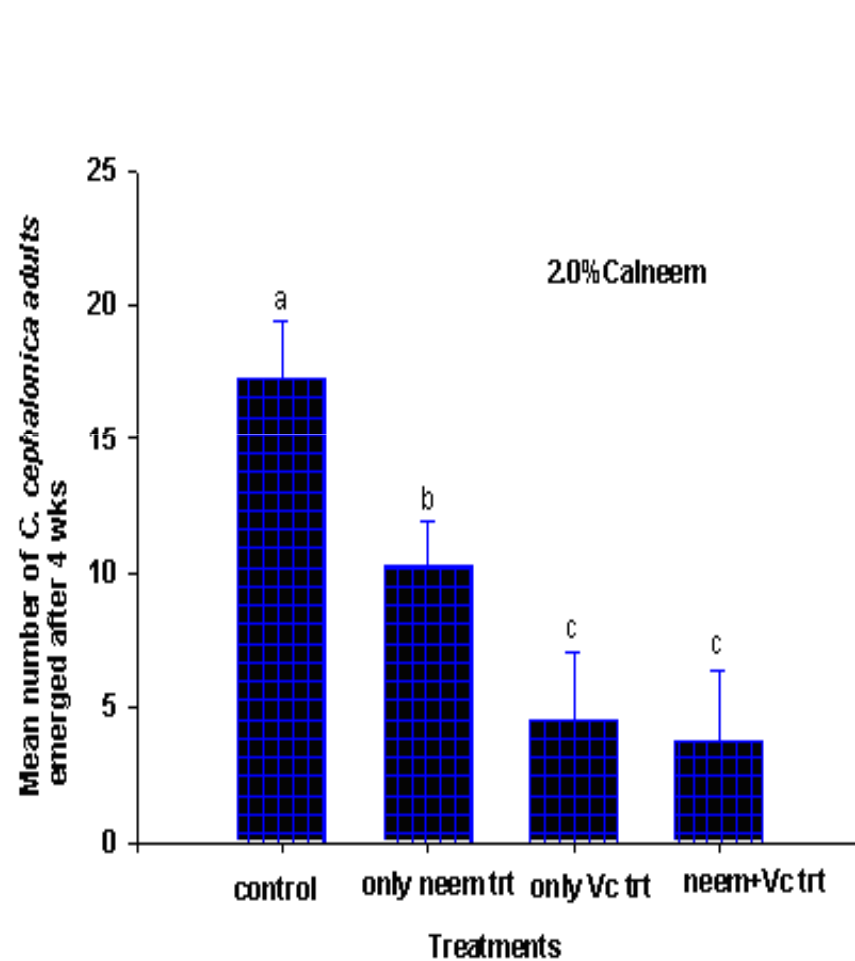


Adult emergence of *C. cephalonica* in jars treated with Calneem oil, *V. canescens* alone or combination of the two and the control



25°C & 65% r.h

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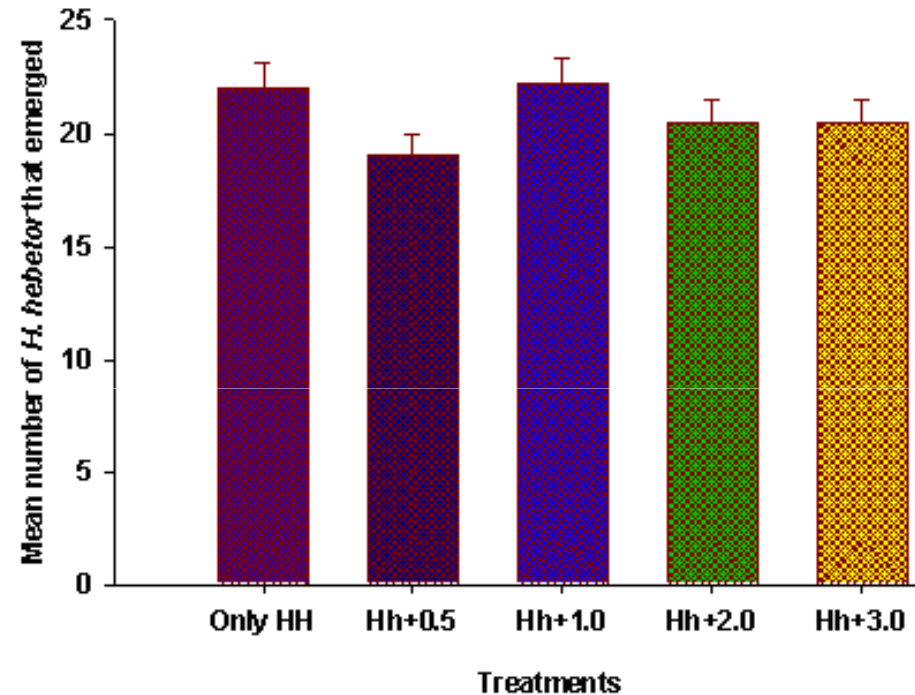
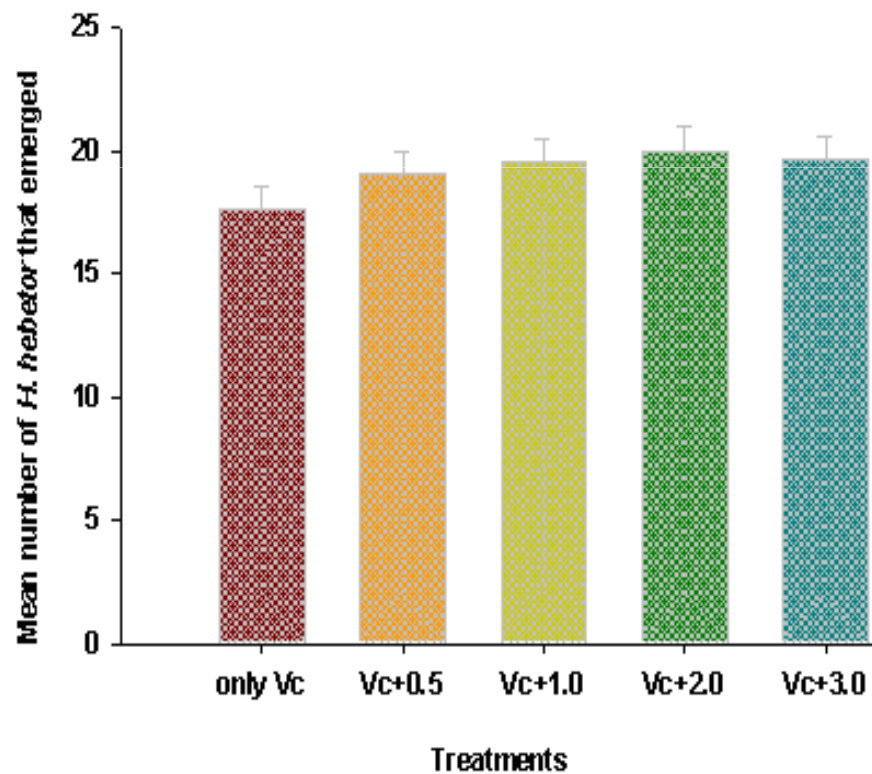


25°C & 65% r.h

Bars denoted by different letter (s) are significantly different (Student Newman Keuls test, $P < 0.05$)



Mean number and SD of *H. hebetor* (Hh) and *V. canescens* (Vc) adults that emerged in rice treated with parasitoids and different doses of CaO (0.5-3.0%)



25°C & 65% r.h

Study Conclusions

- ◆ CalO was toxic to eggs, larvae, pupae and adults of *T. castaneum*
- ◆ Highly repellent to *T. castaneum* with an overall repellency in the range of 80-100%.
- ◆ Results obtained suggest good potential for the practical use of Calneem oil as grain protectant for stored product pest control.
- ◆ Biological control could significantly reduce pest damage in stored grains and could reduce excessive use of synthetic chemicals to control stored product pests in Ghana.
- ◆ CalO and two parasitoids, *H. hebetor* or *V. canescens* alone or in combination with either of the parasitoid caused highly significant reduction in the emergence of *E. cautella* and *C. cephalonica* in stored rice.

- In general, the integration of CalO and *H. hebetor* or *V. canescens* caused the highest reduction in adult emergence of both moth species
- Minimal impact of CalO on the rate of parasitism, reproduction and survival of *H.h* and *V.c* suggests that the use of CalO should be compatible with inundative releases of *H.h* and *V.c* in an integrated approach to stored product pest management strategies without adverse interactions.

The compatibility of the use of neem products and parasitoids in the stored product environment will offer a more acceptable management option against stored product insect pests



Acknowledgement



RosaLuxemburg Stiftung

supported this research

Thanks to Sylvia Krause and Agnes Paul for technical assistance



Thank you for your attention!

