Figure 7: Texture of the fermented matter of potato powder (PP) supplemented LB medium (w/v) by Btk after 12 h incubation at 37 °C. A: 1%; B: 5% and C: 10%.
Figure 8: Texture of the fermented matter of potato powder (10% w/v) supplemented LB medium by *Btk* incubated at 37 °C. A: 12 h showing thick fermented matter and B: at 24 h the thick medium became a slurry.
**Figure 16:** SDS-PAGE protein profile of the crude supernatant and 40 - 60% ammonium sulphate fraction showing α-amylase. Lane 1: molecular weight marker; Lane 2: crude extract and Lane 3: ammonium sulphate fraction.
**Figure 18**: SDS-PAGE protein profile of the sephadex G-100 column fractions showing α-amylase. Lane 1: molecular weight marker; Lane 2: 39th fraction; Lane 3: 38th fraction and Lane 4: 40th fraction.
Figure 35
Figure 36: Endospore production pattern of *Btk* with jack seed powder (JP), potato powder (PP) and tapioca powder (TP) at 10% (W/V) supplemented LB medium and control as revealed by fluorescent microscope (100x) at 48 h. A. Control (LB) showing numerous endospores; B. 10% of jack seed powder showing very few endospores; C. 10% of potato powder showing big endospores; D. 10% of tapioca powder showing numerous big endospores.
Figure 37: Delta endotoxin production pattern of *Btk* on jack seed powder, potato powder and tapioca powder at 10% (W/V) supplemented LB media as revealed by image analyser at 48 h. A: control (LB) showing vegetative cells with endospores and few crystals; B: 10% of jack seed powder showing vegetative cells with spores and crystals; C: 10% of potato powder showing few vegetative cells with endospores and many crystals and D: 10% of tapioca powder showing vegetative cells with endospores and few crystals.
**Figure 38:** Endospores and crystals produced by *Btk* on PP (10 %) supplemented LB medium on water restricted environment at 24 h. Huge endospores (greenish blue) and crystals (bluish pink) are seen with very few vegetative cells or sporulating cells.
Figure 39: Comparative profile of coupled production of endospores and crystals by *Btk* at different conditions. A: 72 h control (LB) showing few vegetative cells, free endospores (greenish blue) and crystals (bluish pink); B: 48 h 10% of PP supplemented medium showing few vegetative cells, sporulating cells, many free endospores and crystals and C: 24 h 10% PP supplemented medium showing huge endospores (greenish blue) and crystals (bluish pink) are seen with very few vegetative cells or sporulating cells.
Figure 40: Scanning electron micrographic images of delta endotoxin production of *Btk* with jack seed powder, potato powder and tapioca powder (10% w/v) supplemented LB media at 48 h. A: control showing rhomboidal crystal with vegetative cells and spores; B: 10% of jack seed powder showing cuboidal crystals and spores; C: 10% of potato powder showing mostly rectangular large crystals with few spores and vegetative cells and D: 10% of tapioca powder showing few crystals, vegetative cells and spores.
Figure 41: SEM images of Btk-toxin with endospores produced by Btk on PP supplemented LB medium (10% w/v) in water restricted environment. A: vegetative cells at 12 h; B: crystal at 24 h (1000 X) and C: 3 times magnified view of B with clusters of crystals.
Figure 44: Habit of *Aceria guerreronis* (mandari) infested coconut palm with tender nuts showing different stages of infestation by mandari. A: *A. guerreronis* infested coconut palm; B: young buttons of 8 weeks old infested nuts develop triangular creamy white patches; C: buttons of 9 weeks old nut develop 1 to 2 patches and D: buttons of 11 week old nut develop longitudinal cracks.
Figure 45: Coconut button and glass rings used for culturing of *A. guereronis*. A: single 30 days aged button show cap region exposed; B; glass rings used for the culture of mites; C; glass rings attached to the button and sealed with parafilm and D: culture set-up (with 4 buttons) in a tray.
Figure 29: Endospore production pattern of *Btk* on various concentrations (w/v) of potato powder (PP) supplemented LB medium at 36 h as revealed by Image Analyser (scale bar = 50 μM): A: control (LB) showing numerous vegetative cells with swollen sporangia; B: 1% showing vegetative cells with numerous endospores; C: 5% showing few vegetative cells and numerous endospores; D: 10% showing few vegetative cells and endospores; E: 20% showing vegetative cells, swollen sporangia and endospores; F: 30% showing vegetative cells, swollen sporangia and endospores; G: 40% showing vegetative cells, swollen sporangia and endospores; H: 50% showing vegetative cells, swollen sporangia and endospores; I: 60% showing vegetative cells, swollen sporangia and endospores; J: 80% showing vegetative cells, swollen sporangia and endospores and K: 100% showing vegetative cells, swollen sporangia and endospores.
Figure 30: Endospore production pattern of *Bt* on various concentrations (w/v) of potato powder (PP) supplemented LB medium at 42 h as revealed by Image Analyser (scale bar = 50 μM): A: control (LB) showing numerous vegetative cells with swollen sporangia; B: 1% showing vegetative cells with numerous endospores; C: 5% showing vegetative cells and numerous endospores; D: 10% showing vegetative cells and numerous endospores; E: 20% showing vegetative cells, swollen sporangia and endospores; F: 30% showing vegetative cells and numerous endospores; G: 40% showing vegetative cells and numerous endospores; H: 50% showing few vegetative cells and endospores; I: 60% showing vegetative cells and numerous endospores; J: 80% showing few vegetative cells and numerous endospores and K: 100% showing few vegetative cells and numerous endospores.
Figure 31: Endospore production pattern of *Btk* on various concentrations (w/v) of potato powder (PP) supplemented LB medium at 48 h as revealed by Image Analyser (scale bar = 50 μM):  
A: control (LB) showing vegetative cells with sporangia;  
B: 1% showing vegetative cells with numerous endospores;  
C: 5% showing vegetative cells and numerous endospores;  
D: 10% showing numerous vegetative cells and endospores;  
E: 20% showing vegetative cells and numerous endospores;  
F: 30% showing vegetative cells, swollen sporangia and numerous endospores;  
G: 40% showing vegetative cells and numerous endospores;  
H: 50% showing vegetative cells and numerous endospores;  
I: 60% showing few vegetative cells and numerous endospores;  
J: 80% showing few vegetative cells and numerous endospores and  
K: 100% showing few vegetative cells and numerous endospores.
Figure 32: Endospore production pattern of *Btk* on various concentrations (w/v) of potato powder (PP) supplemented LB medium at 60 h as revealed by Image Analyser (scale bar = 50 µM): A: control (LB) showing numerous vegetative cells with swollen sporangia; B: 1% showing numerous vegetative cells with endospores; C: 5% showing few vegetative cells with endospores; D: 10% showing very few vegetative cells with endospores; E: 20% showing very few vegetative cells with swollen sporangia endospores; F: 30% showing few vegetative cells with endospores; G: 40% showing few vegetative cells with endospores; H: 50% showing vegetative cells with swollen sporangia and endospores; I: 60% showing vegetative cells with swollen sporangia and numerous endospores; J: 80% showing vegetative cells and numerous endospores and K: 100% showing few vegetative cells and numerous endospores.
Figure 33: Endospore production pattern of *Btk* on various concentrations (w/v) of potato powder (PP) supplemented LB medium at 72 h as revealed by Image Analyser (scale bar = 50 μM): A: control (LB) showing numerous vegetative cells with sporangia and endospores; B: 1% showing few vegetative cells with numerous endospores; C: 5% showing very few vegetative cells and numerous endospores; D: 10% showing few vegetative cells and numerous endospores; E: 20% showing vegetative cells with swollen sporangia and numerous endospores; F: 30% showing few vegetative cells with swollen sporangia and numerous endospores; G: 40% showing very few vegetative cells and numerous endospores; H: 50% showing few vegetative cells and numerous endospores; I: 60% showing very few vegetative cells and endospores; J: 80% showing very few vegetative cells and numerous endospores and K: 100% showing very few vegetative cells and numerous endospores.
Figure 34: Endospore production of Btk with JP supplemented LB medium (10% w/v) and control (LB) as revealed by image analyser (scale bar = 50 μM): A: 12 h control (LB) showing numerous vegetative cells with sporangia; B: 10% of JP at 12 h showing few vegetative cells; C: 24 h control (LB) showing numerous vegetative cells with sporangia; D: 10% of JP at 24 h showing vegetative cells with swollen sporangia; E: 36 h control (LB) showing numerous vegetative cells with swollen sporangia; F: 10% of JP at 36 h showing few vegetative cells with sporangia; G: 48 h control (LB) showing numerous vegetative cells with sporangia; H: 10% of JP at 48 h showing vegetative cells with endospores; I: 60 h control (LB) showing numerous vegetative cells with swollen sporangia; J: 10% of JP at 60 h showing vegetative cells, sporangia and endospores; J: 72 h control (LB) showing vegetative cells and endospores and K: 10% of JP at 72 h showing numerous endospores.
Figure 35: Endospore production of Btk with TP supplemented LB medium (10% w/v) and control (LB) as revealed by image analyser (scale bar = 50 μM). A: 12 h control (LB) showing few vegetative cells with sporangia; B: 10% of TP at 12 h showing numerous vegetative cells with sporangia; C: 24 h control (LB) showing numerous vegetative cells with sporangia; D: 10% of TP at 24 h showing vegetative cells with sporangia; E: 36 h control (LB) showing numerous vegetative cells with sporangia; F: 10% of TP at 36 h showing few vegetative cells with sporangia; G: 48 h control (LB) showing numerous vegetative cells with swollen sporangia; H: 10% of TP at 48 h showing vegetative cells with swollen sporangia; I: 60 h control (LB) showing numerous vegetative cells with swollen sporangia; J: 10% of TP at 60 h showing vegetative cells, sporangia and endospores; K: 72 h control (LB) showing vegetative cells and endospores and L: 10% of TP at 72 h showing vegetative cells and endospores.
Figure 43 A: SDS-PAGE profile of *Btk*-toxin proteins purified from 48 h PP LB medium with or without starch. Lane 1: molecular weight marker; Lane 2: control showed 4 bands with approximate molecular weights of 18 kDa, 30 kDa, 50 kDa and 66 kDa; Lane 3: jack seed powder supplement showed 4 bands with approximate molecular weights of 18 kDa, 30 kDa, 50 kDa and 66 kDa; Lane 4: potato powder supplement showed 3 bands with approximate molecular weights of 18 kDa, 30 kDa and 50 kDa and Lane 5: tapioca powder showed a single band with approximate molecular weight of 60 kDa. This figure reveals that various shape of the crystals are dependent on the growth medium, which in turn are related to molecular weights as revealed by SDS-PAGE profile.

Figure 43 B: SDS-PAGE profile of the *Btk*-toxin proteins purified from 48 h fermented PP supplemented LB medium. Lane 1: molecular weight marker, Lane 2: sample from PP supplemented medium.
Figure 46: Detailed life cycle of *A. guereronis* under culture conditions. A: first day showing adult mites; B: second day showing adult mites with eggs (1 mite with one egg at inset); C: fourth day showing adult mites with numerous eggs (1 mite with 7 eggs at inset); D: sixth day showing first nymph (one first nymph at inset); E: eighth day showing first and second nymphs (one second nymph at inset); F: tenth day showing new adult mites and second nymphs (one adult mite and one second nymph at inset).
Figure 48: Toxicity assay using dry and powdered raw fermented 10% (w/v) PP supplemented LB medium (48 h) containing mixture of endospores, Btk crystals and substrate (µg/cm²). Treatment was made on the mites after 24 h observation of transferred mites in the culture set-up. Photographs were taken after 24 h treatment.

A: control mites treated with sticky uninoculated 10% (w/v) PP supplemented LB medium - here most of the mites were alive and active (above 90%) (two adult mites at inset); B: mites treated with 1.25 fermented matter – here some mites are alive but less active (about 20%) (one active mite with 2 dead mites at inset); C: mites treated with 1.88 fermented matter - here all mites were dead (3 dead mites at inset), D. mites treated with 2.5 fermented matter – here all mites were dead (2 dead mites at inset); E. mites treated with 3.13 fermented matter – here all mites were dead (5 dead mites at inset); F. mites treated with 3.73 fermented matter – here all mites were dead (3 dead mites at inset). From this, it is clear that application of 1.88 µg raw powdered fermented matter per cm² is enough to combat A. guerreronis. In fact, original concentration of the Btk-toxin would be very little in the crude feed.