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Full Length Research Paper

Evaluation of the insecticidal activity of *Fusarium solani* and *Trichoderma harzianum* against cockroaches; *Periplaneta Americana*

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Microbial control is one possible option to reduce cockroaches' populations. On searching for microbial control of cockroaches, a laboratory study was carried out to investigate the effect of spore suspension and metabolites of both fungi *Trichoderma harzianum* and *Fusarium solani* against cockroaches; *Periplaneta americana*. In this study we determined the pathogenicity of both spore suspension and metabolites of both fungi. Spore suspension was applied to the roaches either as a food additive or as a spray. Although both fungi have insecticidal effect on the cockroaches but *F. solani* fungi revealed higher mortality rate than *T. harzianum*. Both application techniques were effective but spraying showed relatively high mortality rate than incorporation of spores as a food additive. Comparing fungal metabolites with the fungal spores, fungal metabolites for both fungi were more effective than the fungal spores. Insecticidal effect of both agents and their metabolites were discussed.

Key words: *Trichoderma harzianum, Fusarium solani*, cockroaches, biocontrol, insect-fungi interaction.

INTRODUCTION

Cockroaches live everywhere; spend most of their time in cracks and crevices. They are found in large commercial buildings such as restaurants, bakeries, grocery stores, food processing plants, hospitals, etc. where cockroaches usually infest food-storage and food-preparation areas, basements and steam tunnels. They can passively transport microbes on their body surfaces including those that are potentially dangerous to humans, particularly in environments such as hospitals (Elgderi et al., 2006). They have been shown to be linked with allergic reactions in humans (Kutrup, 2003). These allergens have been found to be linked with asthma (Kang et al., 1979; Kang et al., 1996).

American cockroaches may transmit bacteria that cause food poisoning (*Salmonella* spp. and *Shigella* spp.). German cockroaches are believed to be capable of transmitting disease-causing organisms such as *Staphylococcus* spp., *Streptococcus* spp., hepatitis virus and coliform bacteria. They also have been implicated in the spread of typhoid and dysentery.

Toxic chemicals usually used for cockroaches control but it can be a serious health risk to the local population and the environment (Gerling, 1990). The environmental and pest-resistance problems associated with intensive use of chemical pesticides has stimulated studies on integrated pest management strategies in which biological control may play a significant role (Gerling, 1990; Heinz, 1996; Nordlund and Legaspi, 1996; Gerling et al., 2001; Naranjo, 2001).

There is increasing interest in the exploitation of fungi for the control of invertebrate pests, weeds and plant diseases as evidenced by the number of commercial products available and under development. The field of

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biocontrol is a burgeoning industry aimed at a more focused, less harmful approach to pest management. This industry has recently homed in on the usage of fungal pathogens to control pest insect populations, specifically agricultural pests (Hajek, 2004). Fungal pathogens have long preyed on insects, claiming millions of victims yearly and all over the world. An array of pathogenic fungal genera can infect a broad range of insects.

The objective of this research is to focus on the potential of fungi as biocontrol agent. The aim was to screen the insecticidal ability of both *Trichoderma harzianum* and *Fusarium solani* fungi either as spores or metabolites using different application methods toward the cockroaches under laboratory conditions.

MATERIALS AND METHODS

Trichoderma harzianum and *Fusarium solani*

T. harzianum was isolated from soil and maintained on potato agar slants (PDA). *F. solani* was isolated from cadaver of an insect and was also maintained as PDA slants. Both fungi were identified by the laboratory of phytomycology at faculty of Science, Suez Canal University.

Insecticidal activity of *Trichoderma harzianum and Fusarium solani* against *Periplaneta americana*

Cockroaches were reared in perforated boxes (cages). Each box containing 10 adults were feed on bread. Boxes were divided into two groups. Each group was subdivided further into four subgroups. Each subgroup was treated with spore suspension ($6 \times 10^6/ml^{-1}$). *T. harzianum* or *F. solani* fungal spores were applied by two techniques, either in the form of spray or introduced as food additive mixed with bread as a carrier.

All boxes were incubated at room temperature for five days; thereafter the mortality rate was calculated. The experiment repeated three times at different seasons. In another group, the filtrates of both fungi were sterilized by Millipore filteration and sprays onto cockroaches in order to test the toxicity of metabolites. The control was autoclaved filtrates. A mixture of metabolites and spore suspensions was also used.

Effect of spore suspension concentration of fungi on *Periplaneta americana*

From the previous experiment, both isolate namely: *T. harzianum*, *F. solani* was retested against *Periplaneta American in vitro*. Three concentrations of spore suspension (5×10^7, 5×10^6 and 1×10^5 ml) were used as spray. Percentage of mortality was measured after 12, 24, 48, 72 and 96 h.

Effect of the application method of fungi on its insecticidal activity

Ten groups of cockroaches were prepared to assess the effect of the application method on the insecticidal activity of *T. harzianum* and *F. solani*. The first group (Ts) was sprayed with 5×10^6 spore/ml of *T. harzianum* while in the second group (T0) the spore suspension of *T. harzianum* was added as a food additive to the crushed bread at zero time and third group (T-7) sterilized bread inoculated with *T. harzianum* 7 days before the beginning of the experiment. Fourth group (TM) received *Trichoderma* mats harvested from 7 days old broth culture. The fifth group (TB) was treated with pure broth containing *Trichoderma* metabolites. These five treatments were repeated with the other fungus *F. solani.* Percentage of mortality was measured daily for 5 days.

Measurement of the lytic activity of *Trichoderma and Fusarium*

The lytic activity of *T. harzianum* and *F. solani* was determined according to Reissig et al. (1959) and Monreal and Reese (1969).

Chitinolytic activity

Both isolates were grown in 50 ml basal medium dispensed in 250 ml Erlenmeyer flaks. The basal medium as described by Sherief et al. (1991) had the following composition g/L dist. water, 10 colloidal chitin, 3 yeast extract, 0.5 KCl, 0.5 $MgSO_4.7H_2O$ and 1 K_2HPO_4. The pH was adjusted to 5. The flasks were inoculated with 1 ml aliquot of spore suspension of 7 day's old culture. The inoculated flasks were incubated on a rotatory shaker for 7 days. The broth culture of each flask was filtered through Whatman No.1 filter paper to separate the mycelium pellet from the culture filtrate. The filtrate was then centrifuged at 4000 rpm for 15 min in a cooling centrifuge. The clear supernatant was considered as the crude enzyme preparation. The reaction mixture contained 1 ml of colloidal chitin (1 g suspended in 100 ml citrate- phosphate buffer pH 5) and 1 ml of crude enzyme preparation. The mixture was incubated for 1 h at 50°C.

The amount of reducing sugar released was determined by the method of Reissig et al. (1959) at 585 nm by using *p-Dimethylaminobenzaldehyde* (DMAB) reagent, pure N-acetyl glucosamine was proceeded in the same way to establish a standard curve. One unit of enzyme activity will produce 1 µmol of N-acetyl glucosamine under these conditions. The protein content of enzyme preparation was determined according to Lowry et al. (1951). 1 ml of the crude filtrate was diluted 20-fold with distilled water. Then, 1 ml of lowry reagent was added to tube. Tube was mixed well and incubated at room temperature for 20 min. Subsequently, with rapid and immediate mixing, 0.5 ml Folin Ciocalteu's reagent was added to all tubes.

After 30 min of color development at room temperature, the absorbances of tested filtrate and standard against blank water were read at 750 nm within 1 h. Total protein concentration was calculated from the standard curve created by using different concentrations of bovine serum albumin (BSA) as standard.

Proteolytic activity

The isolate *T. harzianum* was grown in 100 ml flasks containing 25 ml. Czapek's broth without any nitrogen source but substituted with 1% gelatin. The broth was prepared in citrate phosphate buffer with pH 4.6, inoculated with 1 ml of *Trichoderma* spore suspension and incubated at 25°C for 5 days on shaker. The growth medium was filtered through Whatman No. 1 filter paper followed by sterilization through 0.22 µm millipore filter.

The activity was measured by Spectrophotometer according to Monreal and Reese (1969). Briefly 1 ml of 2% casein solution in phosphate buffer (*M*/20, pH 7.0) was incubated at 50°C with 1 ml of crude enzyme, appropriately diluted with buffer. After 1 h, 2 ml of protein precipitant solution (0.1 *M* trichloroacetic acid + 0.2 *M* sodium acetate) were added, shaked well and the tube was then placed at 40°C for 20 min. The mixture was centrifuged and filtered through Whatman No.1 paper. To 1 ml filtrate, 5 ml of Na_2Co_3 (0.4

M) were added and left for 20 min at room temperature. 1 ml of Folin- Ciocalteau reagent (1 N) was added to the mixture and left for 30 min.The color intensity was measured at 550 *n*m. Hydrolysis activity was estimated from standard curve of L-tyrosin (Lowery et al., 1951). 1 unit of proteolytic activity produces the equivalent of 1 µmol L-tyrosin under the aforementioned conditions.

Data analysis

Data of insect mortality caused by different fungi species and different methods of application were tabulated and analyzed using two ways ANOVA-SPSS (analysis of variance). The differences between the two fungi treatment and the difference between different application methods were tested.

RESULTS

In the first experiment, reduction of cockroaches' population due to fungal treatment by *T. harzianum* or *F. solani* were 100 and 60% respectively at 24 h and reached 100% at the day 3 for both fungi (Appendix 1a and b). Mortality of *Periplaneta americana* caused by both fungi was highly significantly different than control treatment (two way ANOVA on mortality, $F_{6, 30}$ = 41.43, *P*<0.0005), but variable by time or among days of treatments (two way ANOVA on mortality $F_{5, 30}$ = 18.297, *P*<0.0005). Cockroaches treated by *T. harizianum*, T0 (day 0) and Ts (spray) caused the highest mortality rate and highly significantly different than control with mortality rates ranging from 100 and 70% respectively and the least effective application method for the same fungi was T7 (day 7) with 50% mortality rate (Appendix 1a).

Comparing both fungi T7 (day 7) and F7 (day 7) treatment using one way ANOVA, there is no significant difference to the control ($F_{1, 11}$ = 2.6, *P*<0.13). Comparing different application method of the same fungi *Fusarium sp*, F0 (day 0) and Fs (spray) caused 60% insect death rather than F7 (day 7) with 10% death (Appendix 1b). There is no significant difference in insect mortality between T0, F0 treatment (one way ANOVA on mortality, $F_{1, 11}$ = 8.2, *P*<0.016. No significant difference between Ts, Fs treatment (one way ANOVA on mortality $F_{1, 11}$ = 0.0335 *P*<0.8).

Comparing mortality rate caused by T treatment, there is no significant difference (one way ANOVA on mortality $F_{2, 17}$ = 4.14, *P*<0.037). Comparing mortality rate caused by F treatment, there is no significant difference (one way ANOVA on mortality $F_{2,17}$ = 3.47, *P*<0.05). In the second experiment, during autumn, the same sets of experiments were conducted to compare the effectiveness of fungal pathogens at different seasonal temperature. Mortality of *P. americana* caused by both fungi was highly significantly different than control treatment (two way ANOVA on mortality, $F_{10,70}$ = 31.187, *P*<0.0005), variable among days of treatments was highly significantly different than control (two way ANOVA on mortality $F_{7, 70}$ = 12.4, *P*<0.0005).

Fusarium sp caused significantly higher mortality rate than *Trichoderma* sp, Fs (spray) was more effective caused 100% death than Ts (spray) with 70% death. F7 caused 90% insect death while T7 caused 80% insect death (Appendix 2b). Both FB and TM caused 100% insect death and TB caused 30% insect death (Appendix 2a). Comparing mortality rate caused by T treatment, there is highly significantly difference than control (one way ANOVA on mortality $F_{5,47}$ = 7.45, *P*<0.0005).

Comparing mortality rate caused by different F treatment, there is highly significant difference than control (one way ANOVA on mortality $F_{5, 47}$ = 15.39, *P*<0.0001). Comparing mortality rate caused by T7, F7 treatment, there is highly significant difference (one way ANOVA on mortality $F_{2, 21}$ = 19.5, *P*<0.0005). There is highly significant difference between T0, F0 treatment (one way ANOVA on mortality $F_{2, 21}$ = 63.66, *P*<0.0005). Comparing mortality rate caused by TM, FM treatment, there is highly significant difference (one way ANOVA on mortality $F_{2, 21}$ = 22.65, *P*<0.0005). Comparing mortality rate caused by Ts, Fs treatment, there is highly significant difference (one way ANOVA on mortality $F_{2, 21}$ = 22.4, *P*<0.0005).

This experiment showed that Fs (spray) was more effective than Ts. T7 caused 80% insect death, F7 caused 90% insect death, T0 caused 90% death while F0 caused 100% death (Appendix 2a and b). When *Trichoderma* was applied as mats (TM), harvested from 7 days old broth culture, TM caused 60% insect death. When the metabolites of *Fusarium sp* were Millipore filtered and applied to the insects, FM caused 100% death. TB caused 30% insect death while that of *Fusarium* FB caused 100% insect death (Appendix 2a).

In experiment three, during winter, using the same applications methods, after seven days of different fungi treatments, mortality of *P. americana* was highly significantly different than control (ANOVA on mortality $F_{10, 50}$ = 15.45, *P*<0.0005). Difference in mortality among days was highly significantly different (two way ANOVA on mortality $F_{5, 50}$ = 18, *P*<0.0005). T7 caused higher mortality than F7. Both T0 and F0 caused 50% insect death, TM caused 40% insect death while FM caused only 20% insect death. TB was not effective causing no mortality while FB caused 50% death (Appendix 3a and b) and Table 1, Figures 1, 2 and 3 demonstrate different fungi treatments on cockroaches at Summer, autumn and winter respectively.

Figure 4, is a clear comparison between different treatment of both fungi on the mortality of cockroaches. In the other experiment to measure chitinase level, TM and FM were found to have chitin lytic effect where the spores grow on chitin and secrete enzymes causing autolysis after 6 days of application. The value of chitinase for *T. harizianum* was 16.9 while that of *F. solani* is 9.5 as in Table 2.

DISCUSSION

The present study revealed that *Fusarium* treatments

Table 1. Comparison between different treatments of both fungi by different application methods on the mortality rate of cockroaches.

Total (%)	Summer (%)	Autumn (%)	Winter (%)	Season/treatment
80	100	90	50	T0
75	60	100	50	F0
40	70	70	10	Ts
80	60	100	60	Fs
85	50	80	90	T7
55	10	90	20	F7
15		30	0	Tb
75		100	50	Fb
50		60	40	Tm
60		100	20	Fm

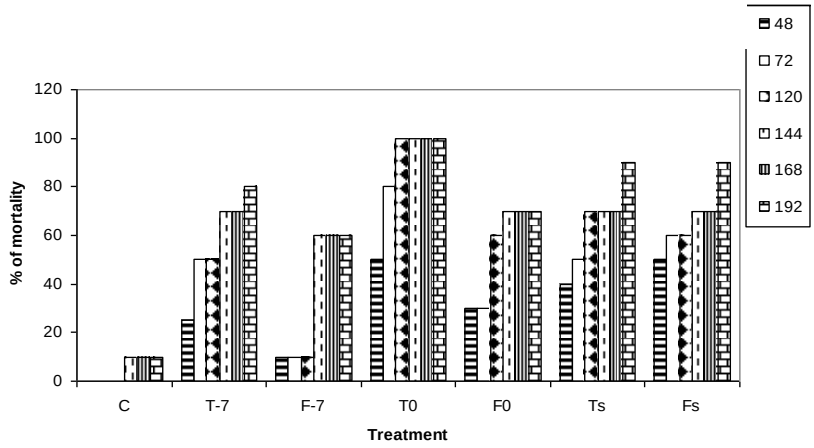

Figure 1. Effect of different fungi species on cockroaches during summer.

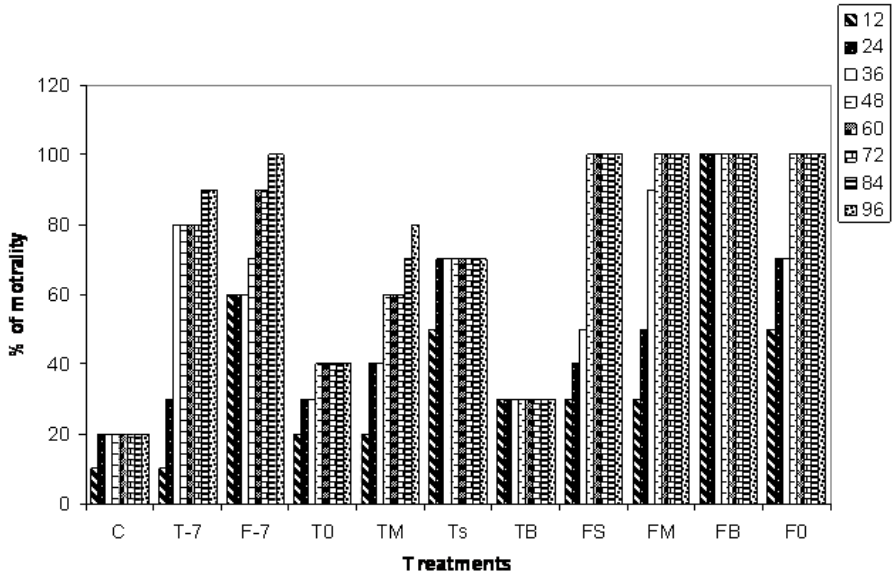

Figure 2. Effect of different fungi species on cockroaches during autumn.

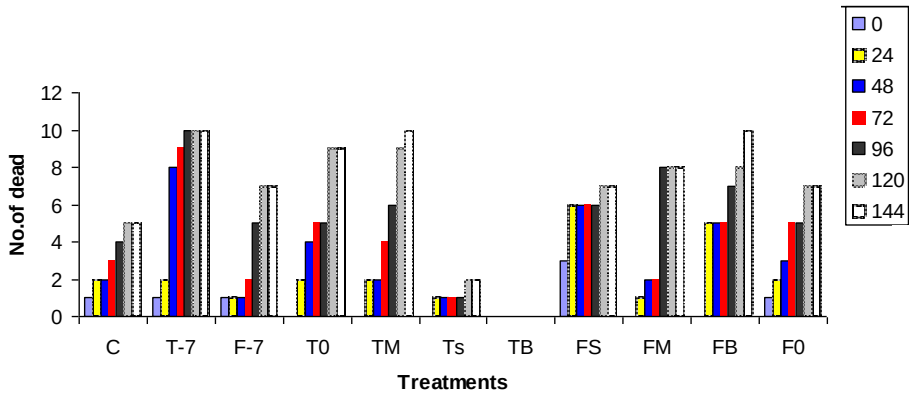

Figure 3. Effect of different fungi species on cockroaches during winter.

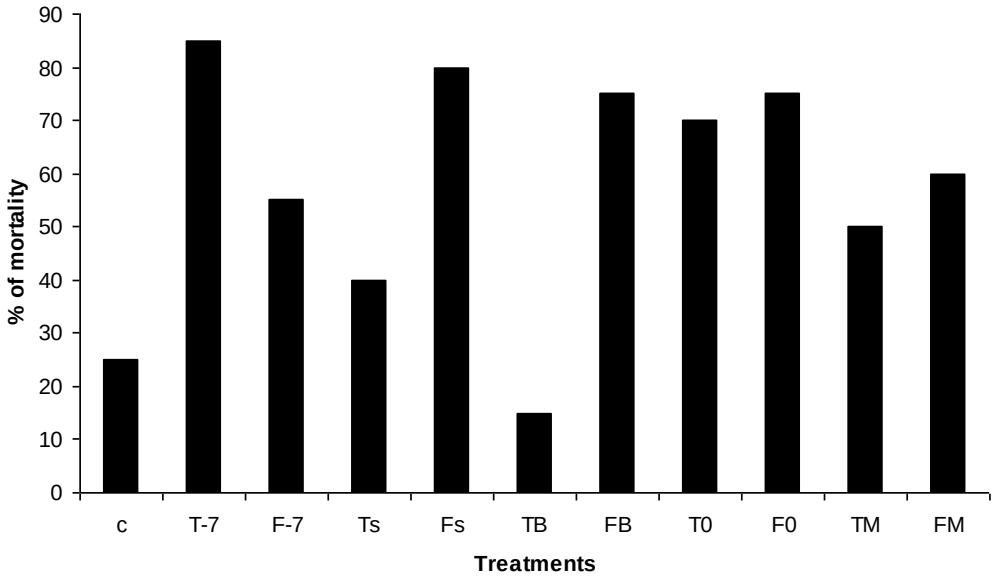

Figure 4. Comparison between different treatment of both fungi on the mortality of cockroaches; F7 is less effect than T7, Fs higher than Ts, FB is higher than TB, F0 higher than T0, FM is higher than TM.

Table 2. Chitinolytic activity of *Trichoderma harzianum* and *Fusarium solani*.

Isolates	Chitinase activity (U/ml)
T. harzianum	16.9
F. solani	9.5

suppress cockroaches' population and was more effective than *Trichoderma*; in agreement with the study under certain conditions where natural entomopathogenic fungi can suppress insect populations and lead to substantial reductions in *Bemisia tabaci* populations (Carruthers et al., 1993; Lacey et al., 2009; Castineiras, 1995). The comparison between different fungi treatments method revealed that the fungus *Fusarium solani* F0 (day 0), F7 (day 7), Fs (spray) caused higher mortality rate than *T. harzianum* T0, T7 and Ts treatment respectively. On the other hand, FB, FM caused significantly higher mortality rate than TB and TM respectively.

Comparing the application methods of fungi, it was obvious that application of spores suspension at zero time to diet T0 was more effective than both direct spray to insect Ts or T7 days old inoculated bread (diet) T7, this is in fact indicated that the matter of application method of the same fungi can affect the effectiveness of the fungal treatment. It was clear that *Fusarium* showed the same trend where the zero day inoculated bread was more effective than both F7 and T7 were the least effective method.

These results indicated that applying the fungi 7 days

before insect start feeding and suppress the fungal effect. According to our results, spraying is the most effective method with high mortality rate in roaches' population. This can be explained by the fact that the effect of the fungi on the insects was through insect spiracle blockage by fungi.

The reason of high mortality rate caused by fungi is thought to be due to closing of insect spiracles by fungal spores during spore germination not due to the fungal toxicity. This suggestion can be confirmed by the fact that spraying method of fungi caused high mortality of roaches' population. The effect of *Fusarium* is suggested to be due to fusaric acid and picolinic acid.

Previous reports of the ability of fusaric acid to inhibit defensive enzymes suggest that these compounds may be important in allowing the producing fungi to be pathogens of insects (Dowd, 1999), cyclic fungal metabolites that can act as chelators or ionophores are produced by many species of fungi that can potentially attack arthropods. Fusaric, picolinic and dipicolinic acids are produced by several species of *Fusarium* (Turner, 1971: Turner and Aldridge, 1983: Bacon et al., 1996) which can also be pathogenic on insects (Claydon and Grove, 1982) or plants (Turner, 1971; Turner and Aldridge, 1983; Bacon et al., 1996; Desjardins, 1992).

The effect of the *Fusarium* may be due to peptide production at insect body. Bcauvericin is a cyclic peptide produced by different species of *Fusarium* (Turner, 1971; Turner and Aldridge, 1983). The lethal effect of the fungus metabolites thought to be due to enzyme inhibition by fungi metabolites.

Some of these fungal cyclic metabolites can be effective enzyme inhibitors. The present results revealed that *Trichoderma* and *Fusarium* found to have chitin lytic effect with *Trichoderma sp* lytic effect were higher than *Fusarium* sp. The fungi grow on chitin and secrete enzymes causing chitin lysis; this is in agreement to most entomopathogenic fungi effect.

Unlike bacteria and viruses that have to be ingested to cause disease, fungi infect insects by direct penetration of the cuticle (Faria and Wraight, 2001). Insect pathogenic fungi possess redundant enzyme systems conferring virulence in terms of capacity to degrade the insect integument (St. Leger et al., 1995). This redundancy obviously reduces the potential for development of resistance based on disruption of enzymes responsible for penetration of the host cuticle (Wraight et al., 2000), which in turn gives the biocontrol agents an advantage to the chemical control.

Repeating the same experiments during autumn revealed that the *Fusarium* effect on mortality rate increased than that of the same fungal treatment during summer. *Trichoderma* treatment did not lose its effect due to any temperature decrease during autumn, but only T7 showed an increase in its effect during autumn, indicated that the temperature increase decrease the effect for only *F. solani* not *T. harizianum*.

Recent studies indicate that, in many instances, high temperature may be a more important factor limiting disease development (Inglis et al., 1996, 2001; Fargues et al., 1997). This is especially significant with regards to control *B. tabaci* which is a key pest in regions with hot and dry climates. Temperature has profound effects on the physiology and development of both the insect host and fungal pathogen through simultaneous effect of host susceptibility and pathogen virulence. One of the insects' responses to fungal diseases is behavioral fever in which insects raise their body temperature as a means of literally toasting a fungal invader. This can be explained as insects are able to elevate body temperatures substantially above ambient and to levels that can inhibit mycosis (Carruthers et al., 1992). The infected flies were actively trying to raise their body temperature in response to infection (Kalsbeek et al., 2001).

TM where the metabolites removed from the treatment and the fungi mass is present is much more effective than the treatment where the metabolite is present TB indicated the fungi mass more effective than the metabolites.

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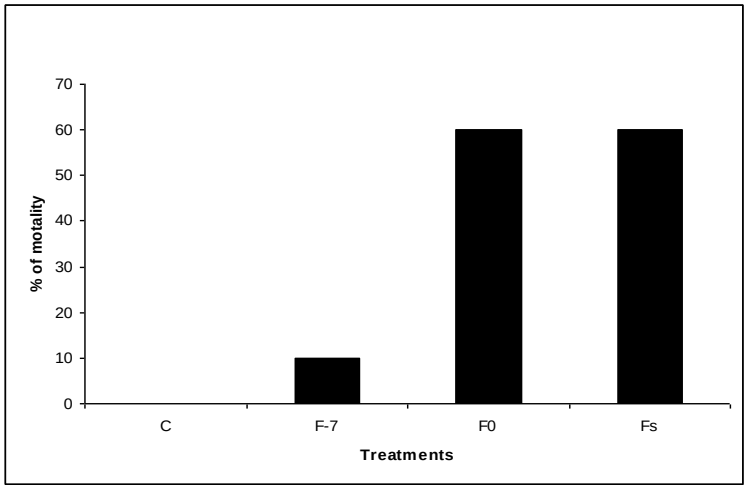
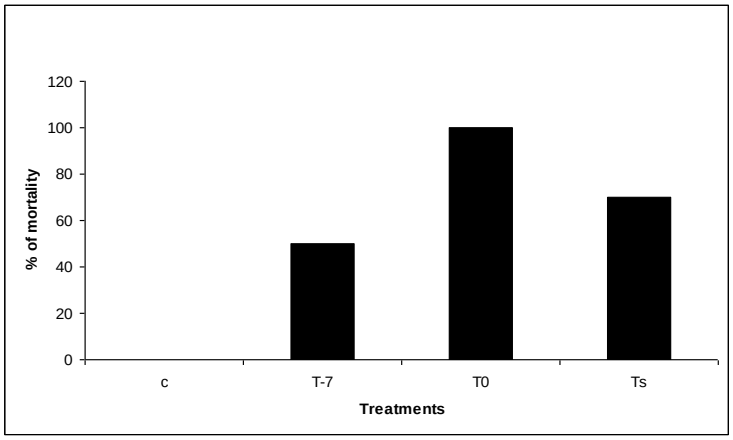
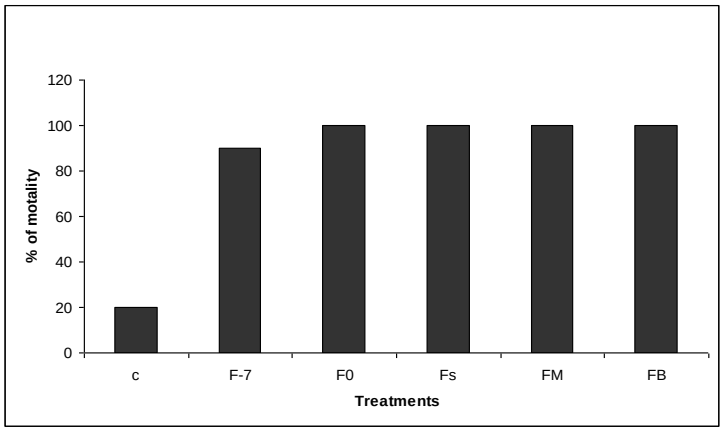


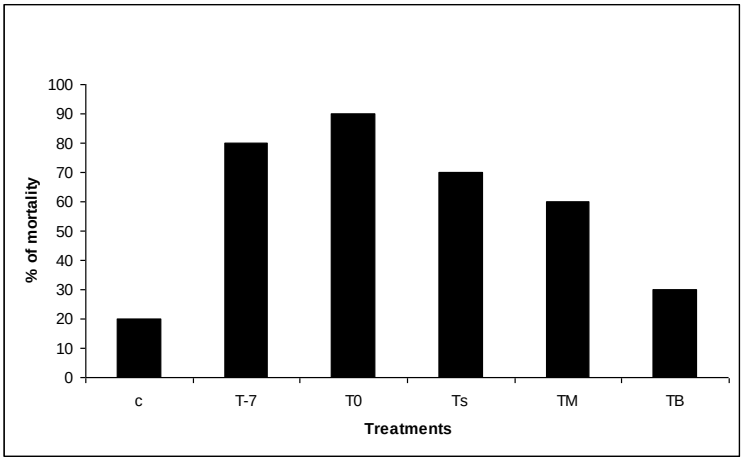
Appendix 1a. Effect of different *Fusarium solani* treatment on cockroaches mortality. F0: F7: Fs.



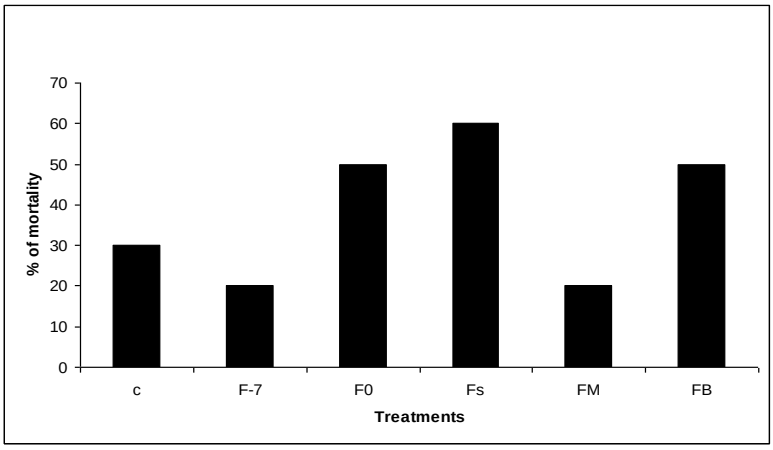
Appendix 1b. Effect of different *Trichoderma sp* treatments on Cockroaches mortality T0, T7, Ts.



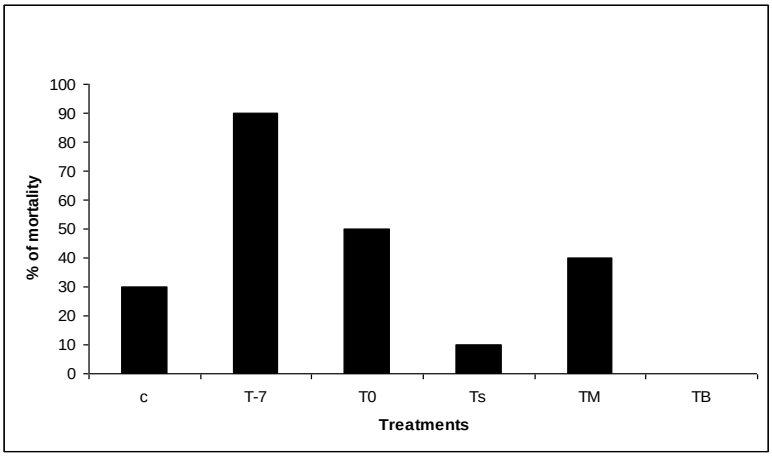
Appendix 2a. Effect of different treatments of *Fusarium solani* on Cockroaches mortality during autumn.



Appendix 2b. Effect of different treatment of *Trichoderma* sp treatment on Cockroaches mortality during autumn.



Appendix 3a. Effect of different treatment of *Fusarium solani* treatment on Cockroaches mortality during winter.



Appendix 3b. Effect of different treatment of *Trichoderma* treatment on Cockroaches mortality during winter.