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Full Length Research Paper

Testing *Paecilomyces lilacinus*, diatomaceous earth and *Azadirachta indica* alone and in combination against cotton aphid (*Aphis gossypii* Glover) (Insecta: Homoptera: Aphididae)

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The efficacy of *Paecilomyces lilacinus* (Thom.) Samson (2.3 × 10^9 conidia ml^{-1}), *Azadirachta indica* (10 mlL^{-1}) and the formulation of diatomaceous earth (PyriSec) (3 gL^{-1}) was tested for the control of cotton aphid (*Aphis gossypii* Glover) (Insecta: Homoptera: Aphididae) both under laboratory and semi-natural conditions. All the tested treatments gave significant control of aphid; however, *P. lilacinus* in combination with Neem showed the best control of aphids in detached leaf bioassay and semi-natural conditions. The applications of *P. lilacinus* and DE showed weak knock down effect on the insect pest. Furthermore, an increasing trend in mortality of aphids was observed in all the treatments with an increase in the time intervals. The results of the study clearly indicated that the *P. lilacinus* may give effective control of the aphids in combination with other eco-friendly control practices.

Key words: *Paecilomyces lilacinus*, diatomaceous earth, *Azadirachta indica*, aphid, cotton, insecticidal efficacy.

INTRODUCTION

Cotton (*Gossypium hirsutum* L.) is attacked by 1326 species of insects and mites that cause severe quantitative and qualitative losses. Among these, aphids and thrips are considered to be the most important insect pests causing 30 to 40% losses at its growing stage (Naqvi, 1976). The cotton aphid *Aphis gossypii* Glover (Insecta: Homoptera: Aphididae) is one of the most common species found on cotton, and is usually present on abaxial surface of leaves. It feeds on phloem, sucks cell sap from leaves and young shoots resulted in distortion, curling, wilting and some time premature defoliation of leaves, that in turn causes reduction of yield both qualitatively and quantitatively. The secretions of honey dew allows associated fungi to grow that causes more than 50 types of diseases in plants due to transmission of viruses (Santos et al., 2004).

Many control strategies for insect pests of cotton have been documented; however, farmer's heavy reliance on pesticides to save the crop from pests resulted in the development of resistance in insects (Hajek and Leger, 1994). The regular and indiscriminate use is another reason of development of resistance in cotton aphid against traditional insecticide like deltamethrin, fenvalerate, monocrotophos, parathion, dimethoate, methomyl and aldicarb (Guilin et al., 1997).

Naturally occurring entomopathogenic fungi (EPFs) considered one of the best alternative to existing chemicals (Hajek and Leger, 1994) and these pathogens

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are found naturally attacking on number of pests (Wraight et al., 2000; Wakil and Ghazanfar, 2010; Riasat et al., 2011). Entomopathogenic fungi such as *Lecanicillium* sp. (Jung et al., 2006), *Beauveria bassiana* (Quesada et al., 2006; Wakil et al., 2011a), *Metarhizium anisopliae* (Wright et al., 2004), *Paecilomyces* (Shia and Feng, 2004) and *Nomuraea rileyi* (Devi et al., 2003; Wakil et al., 2011b) are being used for the control of aphids and other insect pests.

Diatomaceous earths are naturally occurring substances which consists of fossilized skeleton of unicellular diatoms and algae. They possess the high oil absorbing capacity and proved as insecticide (Athanassiou and Kavallieratos, 2005; Athanassiou et al., 2009). (*Azadirachta indica* A. Juss. (L.)) neem belong to family Meliacea is considered as safer bio-pesticide both in conventional and organic agriculture (Koul et al., 1990; Cross et al., 2007). The growth of various insect pests may be altered by the use of oil extracts of seeds and water and ethanol extracts (Montes-Molina et al., 2008). Azadirachtin is one of the potent active ingredients which affect the physiology and the behavior of the various insect pests, mites and nematodes (Ahmed and Grainge, 1985; Smirle et al., 1996; Dhar et al., 1998; Schaaf et al., 2000). Hence, this project was designed to evaluate the insecticidal efficacy of *P. lilacinus*, *A. indica* (Neem) and diatomaceous earth alone and in combination to scrutinize the synergistic interaction against cotton aphid both under laboratory and semi-natural conditions.

MATERIALS AND METHODS

Rearing of *A. gossypii*

A starter colony of aphid was obtained from the fields of Entomological research area and were maintained in the IPM Laboratory, Department of Agri. Entomology, University of Agriculture, Faisalabad, Pakistan on potted cotton plants at room temperature (24 ± 1°C) and 16 L: 8D photoperiod. To synchronize the aphid ages to be used in the bioassays, a group of adult aphids was placed on 8 cm cotton leaf disc in Petri plate containing moistened filter paper and held for 20 h. Adults were removed from the leaf discs and the progeny was then transferred to a potted cotton plant covered with fabric sleeve where they were incubated for 6 days in an insect rearing room before use in bioassays.

Mass-culturing of *P. lilacinus*

The culture of *P. lilacinus* (Thoms.) Samson was provided by the Institute of Plant Protection, Poznan, Poland. It was firstly isolated from the eggs of sugar beet cyst nematode (*Heterodera schachtii* Schmidt) collected from the fields near Toren. The fungus was sub-cultured on Potato Dextrose Agar (Difco™, Becton Dickinson and Company, USA) with the help of sterilized bacteriological loop and the plates were closed by Parafilm at 25 ± 1°C for 14 days. The conidia were harvested using sterilized rubber loop attached to 1 ml borosilicate pipette at the angle of 45°. The scraped material was shifted into sterilized Petri plates and stored at 4°C in refrigerator. The harvested fungal conidia were incorporated in to sterile 0.05% Tween-80 solution and the material was stirred for complete homogeneity. The serial dilutions were prepared and the number of conidia was measured to achieve the 2.3×10^9 conidia ml^{-1} concentration under haemocytometer.

DE and *A. indica* extract

PyriSec® is a mixture of 25% pyrethrum, 3.1% pipronylbutaoxide and 97.5% diatomaceous earth (SilicoSec). Fresh leaves and seeds of Neem were collected from the botanical garden of the University of Agriculture, Faisalabad, Pakistan, and washed with distilled water to remove the dust and other contaminants. They were kept in a muslin cloth for 30 min to drain water. The seeds and leaves were crushed and grinded in the electrical grinder with 100 ml of distilled water to make the paste which was again kept for 72 h. The distilled water was again added to make the solution which was passed through muslin cloth and the extract was stored at 4°C in refrigerator in vials sealed with air tight lid.

Detached leaf-disc bioassay

The detached leaf disc method was adopted and the healthy cotton leaf discs (5 cm in diameter) were placed in 9 cm Petri-plates. The leaf discs were dipped for 5 s in *P. lilacinus* conidial suspension (2.3×10^9 conidia ml^{-1}) for alone and combined treatments. The leaf discs were air-dried in the clean bench and at room temperature for 1 h. The Neem extract (10 mlL^{-1}) was mixed with PyriSec (3 gL^{-1}) and sprayed on the leaf discs with hand sprayer. The leaf discs treated with 0.05% Tween-80 served as control. Each Petri plate with leaf discs was provided with moistened filter paper on the bottom with 1 cm hole on lid covered with fine mesh for aeration. Thirty cotton aphid were released in each Petri plate using camel's hair brush and the Petri plates were then placed in growth chamber maintained at 25 ± 1°C and >70% relative humidity at 16 L:8D photoperiod. The data for mortality was recorded after 2, 4, 6, 8 and 10 days intervals. Each treatment and bioassay was repeated independently for three times. Dead individuals were removed and considered as dead if they did not move when prodded with needle.

Semi-natural conditions bioassay

The cotton plants of var. CIM-496 due to its good yield potential and resistant to CLCuV were grown in pots under semi-natural conditions. The experiments were conducted in the screen house of the Department of Agricultural Entomology, University of Agriculture, Faisalabad, Pakistan. Thirty newly reared adults of cotton aphid were released on the leaves of each potted cotton plant using camel hair brush and allowed to settle for 3 to 4 h. The above said treatments were replicated three times (6-plants per replicate) and both sides of leaves of each plant were sprayed with the help of an automizer. The plants sprayed with 0.05% Tween-80 served as control. The plants were air dried at normal atmospheric conditions for 30 min. The potted plants were covered with fabric sleeve to avoid the movement of adults from one plant to other. The mortality data were recorded after 2, 4, 6, 8 and 10 day. The bioassays were repeated for three times and the individuals considered dead if they did not move when prodded with needle.

Statistical analysis

The mortality data were subjected to statistical analysis using the Minitab 2002 (Minitab, Software Inc., Northampton, MA) and the means were separated by Tukey-Kramer test at $P = 0.05$ (Sokal and Rohlf, 1995).

Table 1. ANOVA parameters for the mortality of *A. gossypii* (total *df* = 119)

Source of variance	df	Leaf disc bioassay		Semi-natural condition	
		F	P	F	P
Replications	3	2.81	0.044	4.24	0.008
Days	4	7080.42	< 0.001	7052.61	< 0.001
Doses	5	1.204	< 0.001	1.104	< 0.001
Days x doses	20	288.78	< 0.001	267.98	< 0.001

RESULTS

Mortality of *A. gossypii* with detached leaf-disc bioassay

The data regarding the effect of different treatments on the aphid in cotton at different intervals showed highly significant difference (Table 1). The maximum mortality 37.67% of aphid was recorded where *P. lilacinus* and Neem was combined. The lowest effect on aphid was recorded 14.50 and 19.25% in *P. lilacinus* and DE, respectively (Figure 1a). *P. lilacinus* in combination with Neem at 4 days resulted to maximum (43.85%) mortality of aphid and the minimum (22.16%) mortality was observed in *P. lilacinus*. However, DE exhibited 25.16% mortality while in combination with *P. lilacinus,* it reaches 35.54% mortality (Figure 1b).

The effect of combination of *P. lilacinus* and Neem was observed to be maximum mortality of aphid at 6 days interval, while DE alone showed minimum effect with 29.85% mortality (Figure 1c). The maximum (61.46%) mortality at 8 days interval was recorded in discs treated with *P. lilacinus* and Neem compared with 54.83% mortality of *P. lilacinus* combined with DE. The minimum 35.83% mortality was recorded in DE treatment (Figure 1d). The mortality (82.89%) was maximum where *P. lilacinus* and Neem was combined, however, the minimum (46.44%) as noted with sole application of *P. lilacinus* (Figure 1e). It is observed that the effectiveness of all the treatments showed an increasing trend up to 10 days of post application.

Mortality of *A. gossypii* with semi-natural conditions bioassay

The effect of *P. lilacinus*, Neem and DE on the population of aphid under semi natural conditions revealed significant difference among treatments (Table 1). The maximum (34.68%) mortality was recorded in *P. lilacinus* and Neem at 2 days interval (Figure 2a). The minimum (12.5 and 15.54%) mortality of aphid was observed with *P. lilacinus* and DE treatment, respectively. *P. lilacinus* and Neem revealed maximum (41.56%) mortality of aphid which was significantly different from all other treatments (Figure 2b). The minimum (22.35%) mortality was recorded in DE; however, *P. lilacinus* with DE and Neem exhibited 31.89 and 29.57% mortality of aphid, respectively. The application of *P. lilacinus* + Neem resulted in maximum (49.50%) mortality which was significantly different with *P. lilacinus* + DE (Figure 2c). The application of Neem showed 37.23% mortality however non significant differences were noted between *P. lilacinus* and DE application with 28.06 and 26.68%, respectively.

The application of *P. lilacinus* and Neem showed maximum (57.35%) mortality, however, DE alone has minimum 32.39% mortality of aphid which showed significant difference with 44.61 and 40.67% mortality with alone application of Neem and *P. lilacinus*, respectively (Figure 2d). The highest mortality (71.45%) was noted with the application of *P. lilacinus* and Neem, while DE and Neem showed 41.23 and 51.32% mortality, respectively. The intermediate response (58.34%) was showed by *P. lilacinus* (Figure 2e).

DISCUSSION

The results of our study clearly indicated that the application of *P. lilacinus* in combination with Neem showed maximum mortality and proved good potential for the control of cotton aphid. Furthermore, *P. lilacinus* in combination with DE showed better results for the control of this notorious insect pest. The present findings are in partial conformity with Santos et al. (2004) who reported that Neem seed powder in highest concentration (1410.0 mg 100 ml^{-1}) was efficient against *A. gossypii* on cotton causing nymph mortality and reducing their survival period and fecundity. Similarly, Stark and Rangus (1994) reported that the molting process of *Acyrthosiphon pisum* (Harris) nymphs was totally interrupted at two concentrations (80 and 100 mg azadirachtin L^{-1}). The nymphs of *Toxoptera citricida* (Kirkaldy) reared on citrus seedlings which were sprayed with Neemix showed the similar response with 0.4 molts (Tang et. al., 2002). The active ingredient (Azadirachtin) actually causes an interruption in ecdysone and juvenile hormone in the insect hemolymph which ultimately affects molting, metamorphosis and reproduction (Mordue and Nisbet, 2000). The pea aphid population was reduced after 3

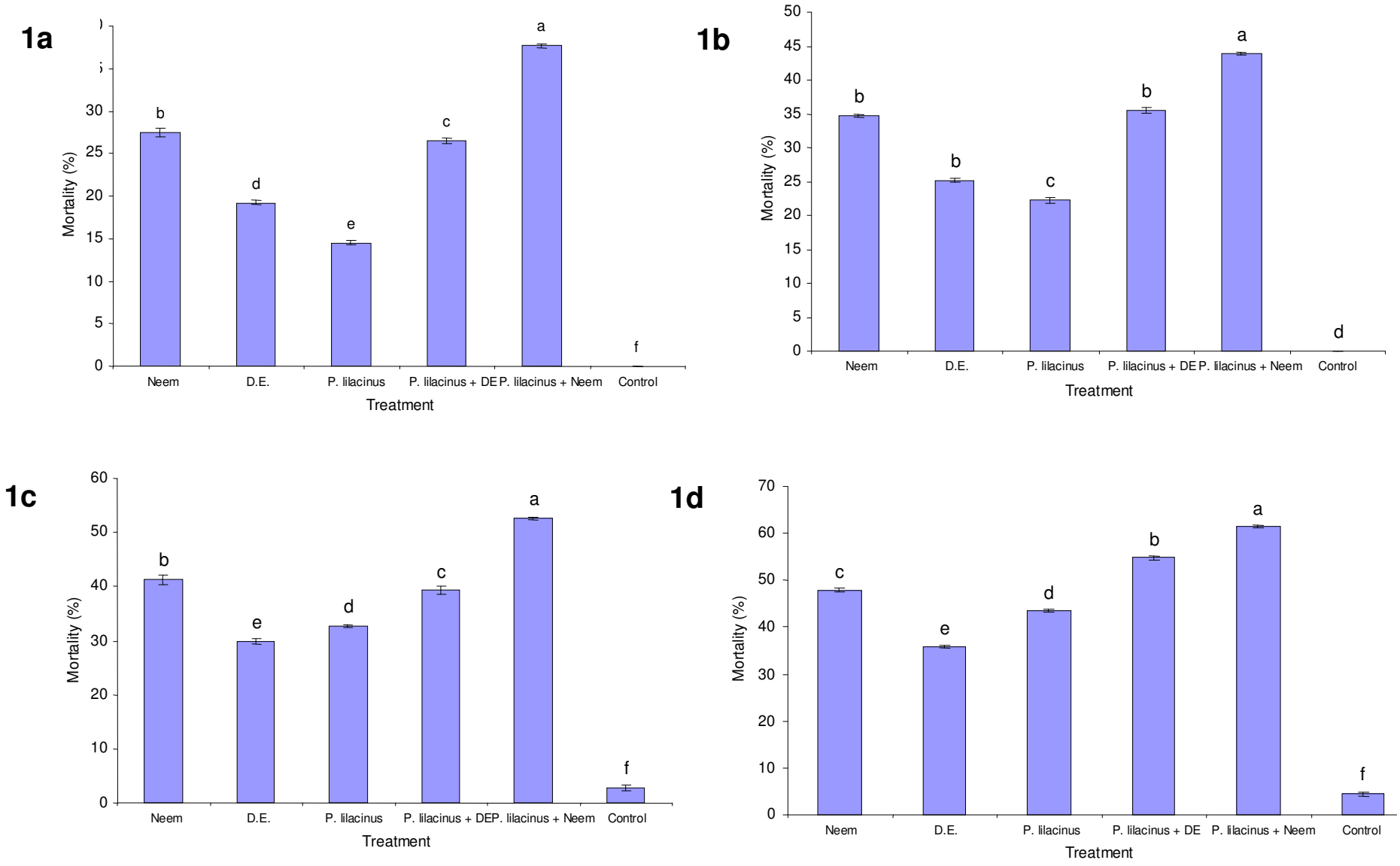
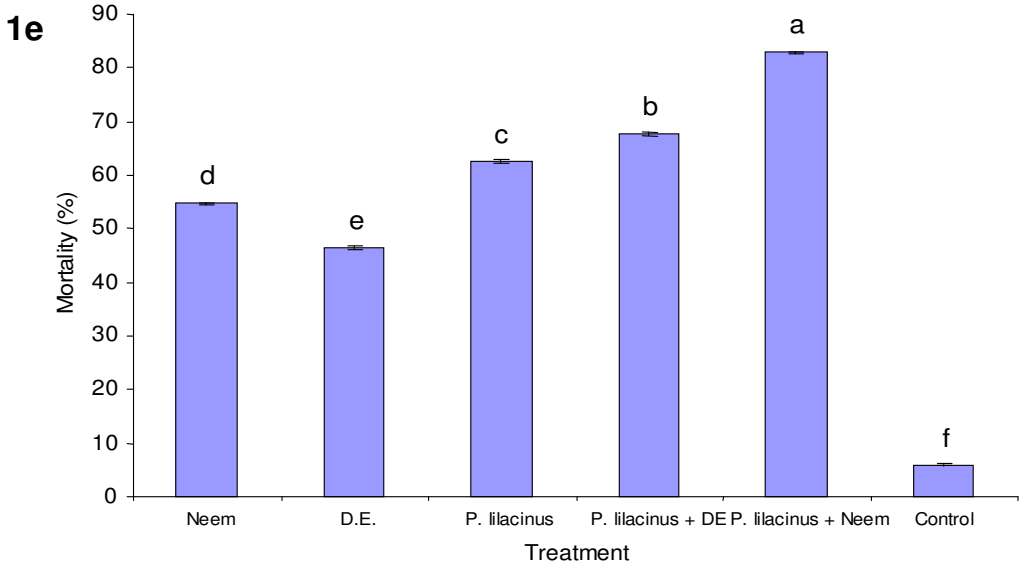


Figure 1. Mean adult mortality (% ± S.E.) of *A. gossypii* exposed for 2 (a), 4 (b), 6 (c), 8 (d), 10 (e) days on detached leaf discs treated with *P. lilacinus* (2.3×10^9 conidia ml^{-1}) with Neem (10 mlL^{-1}) and PyriSec (3 gL^{-1}) (means followed by the same letters are not significantly different at $P = 0.05$).



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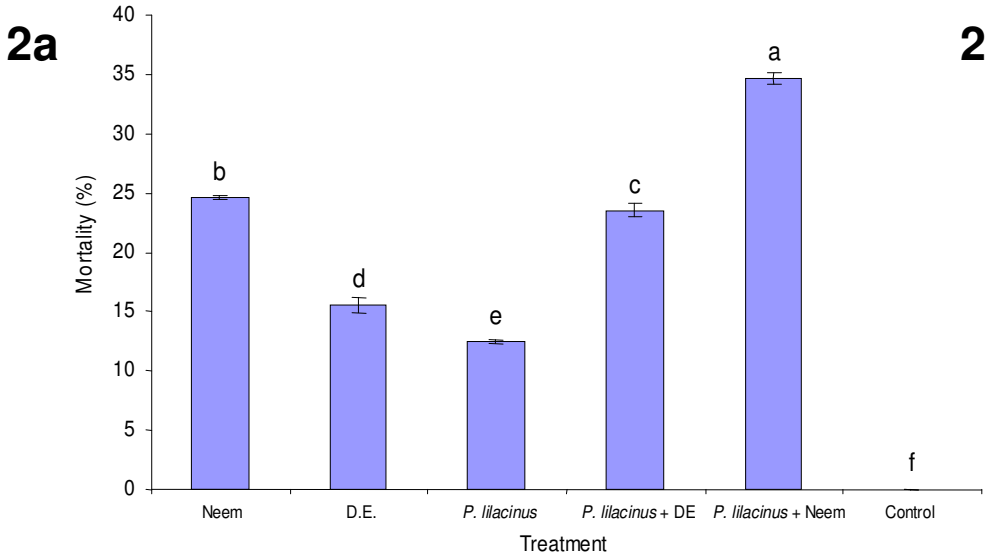

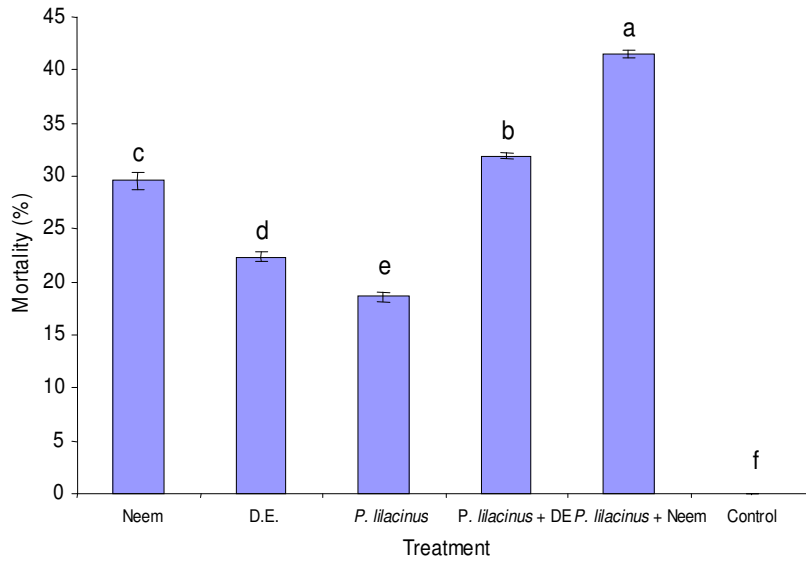

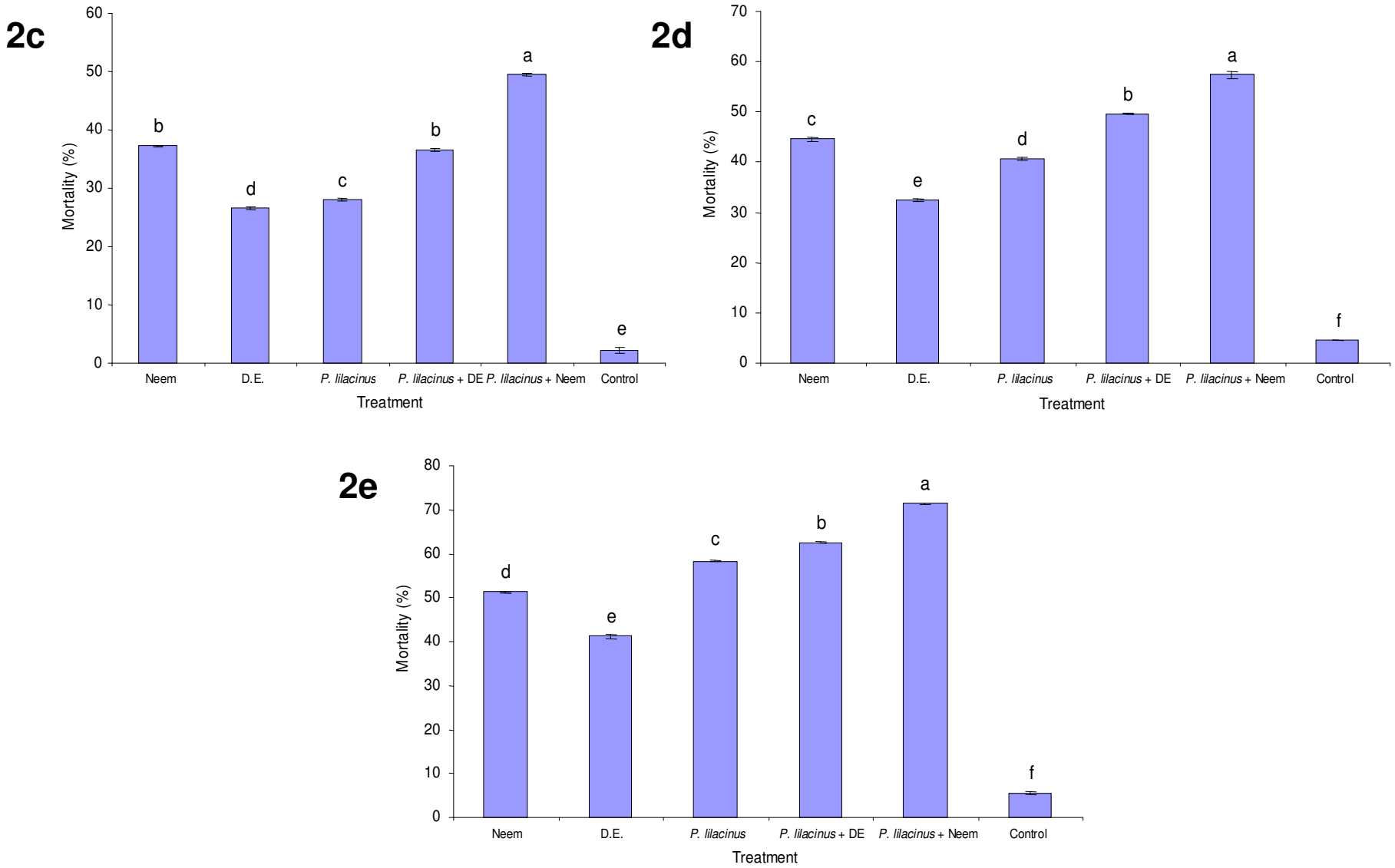

Figure 2. Mean adult mortality (% ± S.E.) of *A. gossypii* exposed for 2 (a), 4 (b), 6 (c), 8 (d), 10 (e) days on cotton under semi-natural conditions treated with *P. lilacinus* (2.3×10^9 conidia ml^{-1}) with Neem (10 ml L^{-1}) and PyriSec (3 gL^{-1}) (means followed by the same letters are not significantly different at $P = 0.05$).

days post treatment up to 50% with the application of azadirachtin compound (Sadeghi et al., 2009). The Neem may also be integrated with DE which presented good results (El-Wakeil and Saleh, 2009) as they reported the combination of Neem with diato-maceous earth against *Myzus persicae* (Sulzer) with best control and no impact on the associated natural enemies.

The entomopathogenic fungi are extensively evaluated for the control of aphid on various crops as Steinkraus (1999) controlled cotton aphid by applying aerial conidia of *Neozygites fresenii.* We observed that *P. lilacinus* was very efficient in controlling aphid both under laboratory and semi-natural conditions even when applied singly or combined with Neem or diatomaceous earth. The significant control of aphid on cotton seedlings was also observed by the application of *Colletotrichum orbiculare* (Russo et al., 1997) which is in line with the present work. Similarly, Kim et al., (2008) tested the effectiveness of the commercial formulation of *Lecanicillium longisporum* (Vertalec) for the control of cotton aphid and reported the noteworthy reduction in the aphid number compared to untreated control. The pathogenicity of different isolates of *Beauveria bassiana*, *Paecilomyces* spp. and *Lecanicillium attenuatum* was evaluated against cotton aphid (Kim and Kim, 2008) where mortality reaches up to 100% after 5 days when treated either with conidia or blasto-spores of the fungus.

Conclusion

In the light of the present study it is concluded that the entomopathogenic fungi may gave better results by replacing the traditional residual insecticides for the control of field insect pests. They may also be triggered by the combination of other safe products like Neem and diatomaceous earth but more research is needed to incorporate them in successful IPM protocols.

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