

The combined use of entomopathogenic fungi and sublethal rate of chemical insecticides or other biological control agents have been proposed as an environmentally and sustainable strategy in the control of locust pests. In this paper, the quarter and the half of the recommended dose of *Metarhizium anisopliae* var. *acridum* ( $\frac{1}{4}$  and  $\frac{1}{2}$  Ma) and the aggregation pheromone (Phenylacetone nitrile: PAN) were applied simultaneously and sequentially to *Schistocerca gregaria* fifth-instar nymphs. In addition, the physiological effects of PAN on locusts were assessed at the behavior, immune response, and biochemical level by evaluating for glutathione-S-transferase (GST), acetylcholinesterase inhibition (AChE), and malondialdehyde accumulation (MDA). Results showed that simultaneous application of PAN and the entomopathogenic fungus exhibited additive interaction. Synergistic interaction was also demonstrated when nymphs were exposed to PAN first, then treated with *M. anisopliae* var. *acridum*. Behavioral bioassay revealed that fifth-instar nymphs avoided the PAN odour and tended to remain away from the stimulus cup. In the choice assay, the pheromone significantly repelled the locusts at 2, 4, and 6 h of exposure which selected the PAN-free arena chamber. Moreover, treated nymphs become hyperactive and disoriented as evidenced by the cumulative distance travelled and the trajectory of locusts during the experiment. Immunological studies showed that PAN significantly decreased the differential haemocyte counts (prohemocytes and plasmatocytes) with a dose-response relationship. Data of biochemical analyzes showed that the PAN

exposure reduced the activity of acetylcholinesterase and induced significantly the glutathione S-transferases and MDA concentration in the desert locust fifth-instar nymphs. Moreover, transcriptomic responses to the PAN exposure were evaluated using gene expression levels of CYP540 and GST. The transcript levels showed an up-regulation in GST expression level particularly in nymphs exposed for 4 and 6 h. A significant increase in CYP450 transcript level was also observed after 2 h of exposure, which decreased significantly after 4 and 6 h. © 2020 Elsevier Inc