The Effects of Kojic Acid and PTU on the Synergistic Antimicrobial Properties of S. bullata & S. frugiperda Hemolymph with Gentamcyin on E. coli

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The purpose of this research was to determine the effects of enzyme inhibitors, kojic acid and phenylthiourea (PTU), on the melanization of hemolymph and its synergistic properties when combined with the gentamycin. The experiment also compared the synergistic properties of S. bullata hemolymph to that of S. frugiperda. A synergistic antimicrobial effect was hypothesized. The minimum inhibitory concentration of gentamycin was used. Three concentrations of hemolymph were used for each species, and each set of three was also combined with gentamycin, and then with PTU and kojic acid, respectively. Two 96-well plates were used, and five controls were tested on both plates. Hemolymph was extracted from pupae and then purified through centrifugation. Concentrations were created, and the solutions and E. coli were pipetted into the plates for a total of 200µl per well. 36 experimental groups and five control groups were tested, with 3 wells per group. The plates were incubated for 26 hours with optical density measured. The results show that synergy occurred between the lowest concentration of S. frugiperda hemolymph and gentamycin and both inhibitors. The use of enzyme inhibitors increased the efficacy of antimicrobial peptides (AMPs) in S. frugiperda hemolymph but decreased it in S. bullata hemolymph. ANOVAs and T-tests indicate statistically significant differences. The enzyme inhibitors show a clear distinction between immune responses in different species, providing a gateway for research in innate immunity and medicine and for identification of previously-unknown AMPs. AMPs from hemolymph in synergistic solutions may combat rising antibiotic resistance.

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