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Database Preparation and Search for Protein Identification

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The mass-spec data were searched against the customized mosquito larvicidal toxin database which included well established larvicidal toxins like Cry1 to Cry73, Bin-A/B, VIP-1/2/3/4, and Mtx-1/2/3. (28). The default search parameters used for the analysis are as follows: (a) trypsin as the proteolytic enzyme with up to one missed cleavage; (b) peptide mass error tolerance of 20ppm; (c) fragment mass error tolerance of 0.1 Da; (d) carb-amido-methylation of cysteine as fixed modification (e) oxidation of methionine as a variable modification. A false discovery rate of 1% was applied while identifying the peptide-spectrum matches. Two criteria used to report a positive find, 1) reported protein/toxin should have least two numbers of peptides in amino acid sequence; and 2) the length of each of the identified peptide sequences using LC-MS has a minimum of 10 amino acids.