BATCH ANALYSIS VALIDATION OF MICROBIAL PESTICIDE USING MICROBIOLOGICAL METHODS

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INTRODUCTION

Batch analysis is useful test to analyze potential risks associated in terms of impurities/contaminating microbes in a product aimed as microbial pesticides. The test also enables the establishment of variation of concentration of the intended microbes in the microbial pesticide product. Batch analysis involves five steps: sample collection, sample preparation, sample testing, data analysis and reporting. The results of batch analysis are followed as per standards set by the Bacteriological Analytical Manual (BAM) and HPFB MFHPB-21 Sept 2005 EN "Enumeration of Staphylococcus aureus in Foods".

Different batches of the commercially available product, that are claimed to contain active microbes at a defined CFU count, are tested for enumeration. Pathogenic or harmful microbial contaminants which could potentially cause adverse effects on human health and can be checked for the presence or absence with the help of microbiological methods.

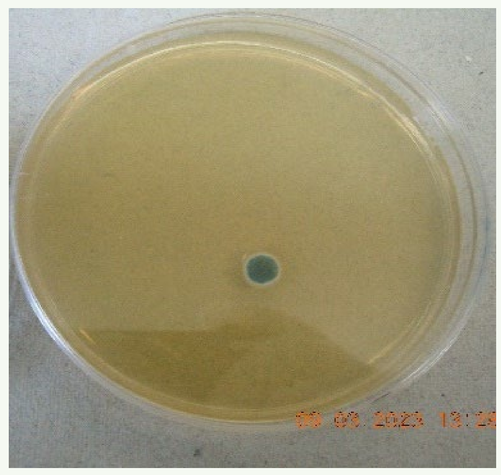

Figure 1: Trichoderma colonies on Potato dextrose agar

MATERIALS AND METHODS

Based on the active organism growth medium and conditions are selected. For microbial impurities/contamination, the particular contaminating organism test can be performed as per BAM guidelines. In the current study we analysed commercial product contain *Trichoderma harzianum* (1×10^9 CFU/g). Two different batches of same product taken and checked active ingredient content of *Trichoderma harzianum* and impurity testing for *Escherichia coli* (as per BAM chapter 4), *Staphylococcus aureus* (As per HPFB MFHPB-21 Sept 2005 EN), *Salmonella* (as per BAM chapter 5), aerobic plate count (as per BAM chapter 3), yeast and mold (as per BAM chapter 18).

For testing of *E.coli*, *S.aureus, Salmonella* and yeast-mold, selective media like eosine methyl blue agar, mannitol salt agar, xylose lysin deoxycholate agar, hektoen enteric agar, dichloran rose bengal chloramphenicol agar, potato dextrose agar, standard plate count agar and Sabroauds chloramphenicol agar were used. The aerobic plate count method and specific biochemical test like Indole , Methyl red, Voges Proskauer , Citrate, Oxidase, Catalase, gram staining, fungal staining used to determined the *E.coli*, *S.aureus, Salmonella* and yeast-mold.

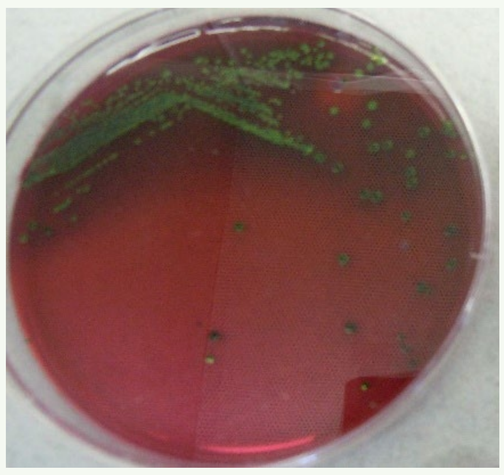

Figure 2: Positive Culture characteristics for *Escherichia coli* on Eoisine Methylene Blue Agar,

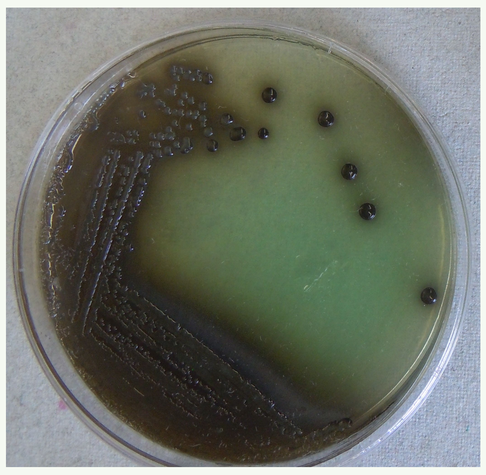

Figure 3: Positive Culture characteristics for *Salmonella* on Bismuth Sulphite Agar

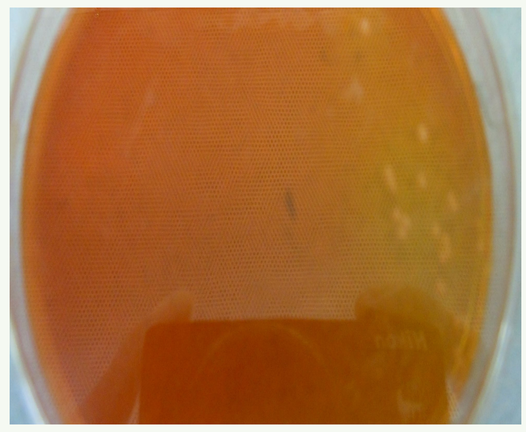

Figure 4: Positive Culture characteristics for *staphylococcus aureus* on mannitol salt agar

Growth Medium	Targated Organism	Result of Positive Culture	Gram Staining	Sample-1 Result	Sample-2 Results
Eosine Methylene Blue agar	Escherichia coli	Green Metallic sheen Observed	Gram-negative pink red color rod shaped bacteria	-ve	-ve
Bismuth Sulphite Agar	Salmonella	Brown or black colonies with or without black center	Gram-negative rod-shaped bacteria	-ve	-ve
Xylose Lysin Deoxycholate agar		Pink colour colonies with or without black center		-ve	-ve
Hektone enteric (HE) agar		Blue green colonies with or without black colou		-ve	-ve
Mannitol Salt Agar	Staphylococcus aureus	yellowish/cream colour colonies	Gram Positive Cocci Shaped	-ve	-ve
Potato dextrose agar	Yeast & Mold	Green Color Colony	-	1.4×10^9 CFU/g	1.1×10^9 CFU/g
Dichloran rose bengal chloramphenicol agar		Yeast, Mold, Fungal Colony	-	-ve	-ve
Sabroauds chloramphenicol agar	Aerobic Plate Count	Fungal Colony	-	-ve	-ve
Standard Plate Count agar		-	-	-ve	-ve
Urease	Biochemical Tests	Purple-red color	-	-ve	-ve
Indole test		Violet Color at surface	-	-ve	-ve
Methyl red test		Diffuse red color	-	-ve	-ve
Voges-Proskauer test		Pink to red color	-	-ve	-ve
Citrate Test		Turbidity	-	-ve	-ve
Oxidase Test		Change in Purple Color	-	-ve	-ve
Catalase Test		Bubble Formation	-	-ve	-ve

RESULTS

The two batches of product, the concentration of *Trichoderma harzianum* (1×10^9 CFU/g) varied showed the variation of 21.4% (Batch 1: 1.4×10^9 CFU/g and batch 2: 1.1×10^9 CFU/g) concentration of *Trichoderma harzianum*. The pure culture of *E.coli*, *S.aureus, Salmonella* and yeast-mold were used to determine the *E.coli*, *S.aureus, Salmonella* and yeast-mold in the two baches of product. No contamination was detected in these two baches.

DISCUSSION AND CONCLUSION

Thus the study helped establishment that the product contained the active microbes *Trichoderma harzianum,* at a CFU count between 1.1 to 1.4×10^9 CFU. The product was free of any contaminating *E.coli*, *S.aureus, Salmonella* and yeast-mold in these batches.

REFERENCES

BAM Chapter 3: Aerobic Plate Count

BAM Chapter 4: Enumeration of Escherichia coli and the Coliform Bacteria

BAM Chapter 5: Salmonella

BAM Chapter 18: Yeasts, Molds and Mycotoxins

HPFB MFHPB-21 Sept 2005 EN Enumeration of Staphylococcus aureus in Foods

FURTHER CONTACT

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