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Studies on the efficacy of bacteria as control agents of mealybugs

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ABSTRACT: The efficacy of *Serratia marcescens, Pseudomonas aeruginosa* and *Bacillus thuringiensis* as control agents of mango mealybugs, *Rastrococcus invadens* Williams, was carried out. Pathogenicity of the bacteria cultures on the live mealybugs were carried out with the aid of an aerial handsprayer using varying concentrations of the microorganisms. All the microorganisms caused the death of the mealybugs but with varying degrees of efficiency. *Bacillus thuringiensis* was more effective than *Serratia marcescens* and *Pseudomonas aeruginosa.*

Key Words: Pest control; Biological control; Mango mealybugs.

Introduction

A lot of insect pests feed on and devour our crops and economic trees, reducing yield and lowering the market value of these crops. One of these insect pests is the mealy bugs which attack our ornamental plants such as Frangipani, Oleander and *Jatropha*. The mango mealy bug, *Rastrococcus invadens* Williams (Hemiptera: Pseudococcidae) occur in many parts of Nigeria and on a variety of crops including sweet orange and guava (Wood and Ambridge, 1996). The white mealy bugs can usually be found feeding along the veins on the lower surface of the leaves. Feeding causes leaves and shoots to become malformed, stunted or necrotic. Defoliation can also occur. Fruits of infested trees are small and malformed. Honey dew produced by the insects encourage the growth of sooty moulds over the foliage and fruits reducing fruit quality and marketability (Wood and Ambridge, 1996).

A reported case of heavy infestation of mango and avocadoes by mealy bugs in Ghana revealed 80% loss from exported mangoes (Agounke et al., 1988). However, it is difficult to assess crop losses caused by mealy bugs in Nigeria and in the entire West African sub-region because the traditional nature of horticulture hampers collection of statistical data.

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Chemical insecticides such as permethrin, dimethoate, carbaryl and cypermethrin have been used to control mealy bugs and other insect pests, but these chemicals have serious drawbacks such as the development of resistant insect strains, toxic residues, workers' safety and increasing costs (Sighamony et al., 1986). These have necessitated research on the use of alternative insect pest control methods. However, biological control of mealy bugs appears to be gaining widespread acceptance as this has led to the identification of a complex array of natural enemies of mealy bugs classified into predators, parasitoids, hyperparasitoids, parasites and pathogens (Matanmi, 1989).

Mealy bugs activities have led to sharp and panicky reactions by people in an attempt to control the pest. Such attempts include trimming of infested plants, felling of infested trees, and cutting of ornamentsl plants (Asgounke et al., 1988). Some microorganisms belonging to the group bacteria, viruses, fungi, protozoans have been used as biocontrol agents (Boman, 1981). The term "microbial insecticides" is defined to include microbial metabolites or entomopoisons produced by microorganisms. Examples include *Bacillus thuringiensis* delta-endotoxin which is very active against many lepidopterous and dipterous larvae; beauvericin, a toxic metabolite of the fungus *Beauveria bassiana* and other entomopathogens which might not produce any toxic metabolites at all but are mass produced, formulated and applied as conventional insecticides (Matanmi, 1989).

Generally, the application of microbial insecticides has been made with equipment and technology developed for chemical herbicides (Ignoffo and Boening, 1970; Kellen et al., 1977; Henry et al., 1978). Increase in insect mortality could be achieved by the inclusion of additives (Kellen et al., 1977).

As of now, microbial insecticides are not in operational use in Nigeria. However, there is a basis for the detection, isolation and subsequent development of naturally occurring entomopathogens. Hence, this work investigated the control of mango mealy bugs using *Serratia marcescens, Pseudomonas aeruginosa* and *Bacillus thunringiensis*.

Materials and Methods

Collection of mealy bugs

Mealy bugs (*Rastrococcus invadens* Williams) were collected inside petri dishes from the surface of mango leaves beside ECWA Chapel in Ilorin, Kwara State, Nigeria.

Test organisms

The test organisms, namely, *B. thuringiensis, S. marcescens* and *P. aeruginosa* were obtained from the culture collection center of the Department of Biological Sciences, University of Ilorin, Nigeria. The cultures were maintained on agar slants at 4°C.

Determination of spray concentrations of the cultures

Three test tubes containing 10 ml each of nutrient broth were prepared and sterilized at 121°C for 15 minutes. The pure cultures of *B. thuringiensis, S. marcescens* and *P. aeruginosa* were transferred differently into nutrient broth with the aid of a wire loop, after which it was mixed, serially diluted up to 10^{-3} and incubated at 37°C for 24 hours. One ml was taken from each of the nutrient broth into sterile petri dishes to which sterile molten nutrient agar was added and incubated at 37°C for 24 hours to determine the viable bacterial counts present in the nutrient broth. The concentrations of *B. thuringiensis, S. marcescens* and *P. aeruginosa* per 10 ml were therefore determined.

Hand spray was used so as to monitor the concentrations of the droplet falling on the mealy bugs. A single spray from the nozzle of the spraying equipment was equivalent to 0.2 ml which was also equivalent to 20 drops when a syringe was used. However, two sprays were used to test for the efficacy of the microbial cultures.

Treatment of leaf surfaces

The experiment was carried out with three small mango seedlings (less than 1 metre tall) per pot. Twenty five insects were put on the leaves of the mango seedlings, five on each leaflet. The insects were allowed to become active and acclimatize for two days. Each leaflet containing these mealy bugs were labeled with the name of the organism proposed to be sprayed on it.

Droplet Application

Droplets were administered to the leaf surfaces by aerial applied sprays. This technique allowed specific concentrations of the three organisms to be applied. There were three pots per treatment, the mango seedlings were set out for each spray treatment in rows of three pots, 1 meter apart. Each of the concentrations (2.2×10^2, 1.4×10^2 and 0.4×10^2) of *B. thuringiensis* on different sets of leaflets that contained 25 mealy bugs, i.e. concentration 2.2×10^2 for 5 leaflets on the first mango seedling in the first pot, concentration 1.4×10^2 for another 5 leaflets on the second mango seedling on the same pot and concentration 0.4×10^2 for another 5 leaflets on the third mango seedling on the same pot. This was repeated for the other two microbes (*S. marcescens* and *P. aeruginosa*) with the remaining two pots that contained three mango seedlings each.

Spraying was performed at right angles to the line of the trees and was directed at the portion of the leaflets. As a precautionary measure during the time of spraying, the insect body was covered with the microbial culture. The trees were laid out inside the laboratory under a 24 hr lighting regime, those for each concentration on a separate table to reduce the possibility of mealy bugs movement between treatments, all at room temperature. The experiment was checked daily and the trees were watered as required. Signs of mortality were observed after the second day of spraying and these were recorded, and further observation done every other day.

Results and Discussion

Figs. 1 – 3 show the effect of different concentrations of bacterial isolates on mortality rate of mealy bugs. The results show that the bacterial isolates were effective in controlling mealy bugs at higher concentrations. At the end of ten days after application, the mortality for mealy bugs was 84%, 96% and 96% for *P. aeruginosa, S. marcescens* and *B. thuringiensis* respectively. *S. marcescens* was close in efficacy to *B. thuringiensis* at all concentrations and same length of exposure to mealy bugs. The mortality of the mealy bugs increased with length of exposure and no death was recorded on the second day of application of low concentration for all the three test organisms.

B. thuringiensis was found to be the most potent agent for the control of mealy bugs. This is probably due to changes in contact rate as a result of changes in droplet concentration which might have produced toxic effects on the mealy bugs. Fast and Regniere (1984) have shown that *B. thuringiensis*-induced mortality sharply decreased with both length of exposure of toxin and dose. The increase in mortality observed in the present study was thought to be caused by a cumulative response of the insects through depletion of regenerative capacity of the mid-gut epithelium.

Most bacteria enter the body cavity through a mechanical injury or with the aid of supporting host physiological factors, e.g. gut, the contributions of which are not yet fully understood. In a number of insects, a petritrophic membrane lines the central part of the intestinal tract. The membrane, if present, protects physically the gut epithelial cells and it differs in various insect species (Reichard et al., 1978). The normal microflora of the gut act as a buffer that maintains appropriate conditions which are of great importance in the insect gut (Alm et al., 1987).

S. marcescens is an insect pathogen, ubiquitous saprophytic bacterium, frequently isolated from many diseased and dead insects. They multiply in the gut of the mealy bugs probably after ingestion to cause disease which eventually lead to the death of the mealy bugs (Fast and Regniere, 1984). Intestinal diseases caused by a disturbance of the gut microflora and subsequent imbalance of the gut metabolism are more frequent than intestinal intoxication which resembles food poisoning (Boman, 1981). Absence of mortality in the mealy bugs at low concentrations of 0.4×10^2 of all the bacteria isolates could be due to immune

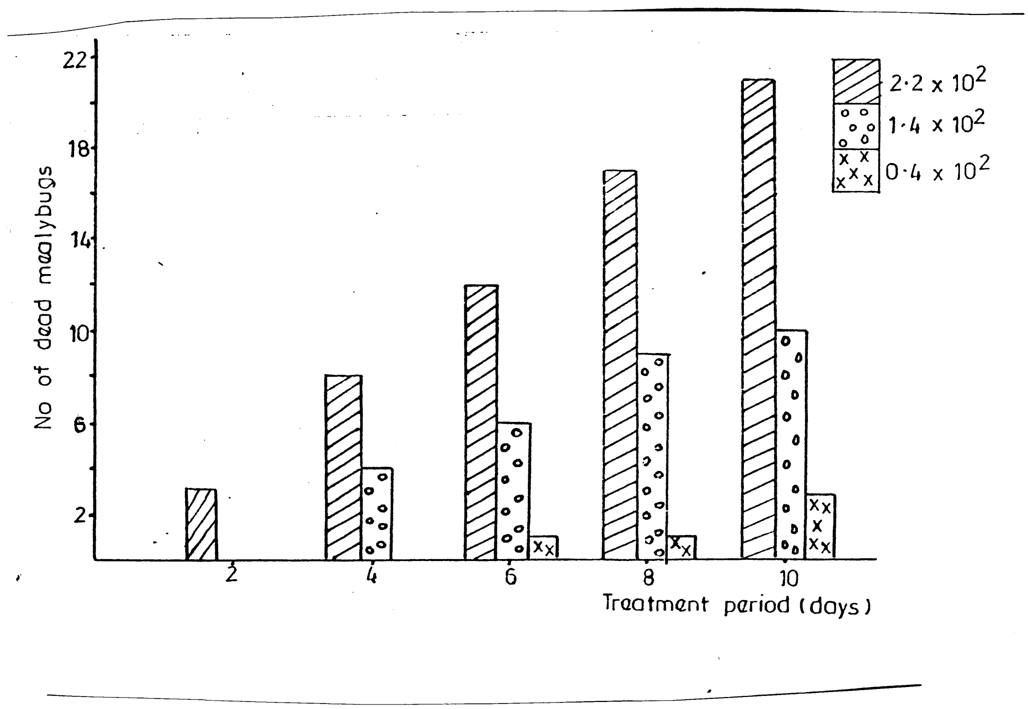

Fig. 1: Effect of different concentrations of *P. aeruginosa* on mortality rate of mealybugs.

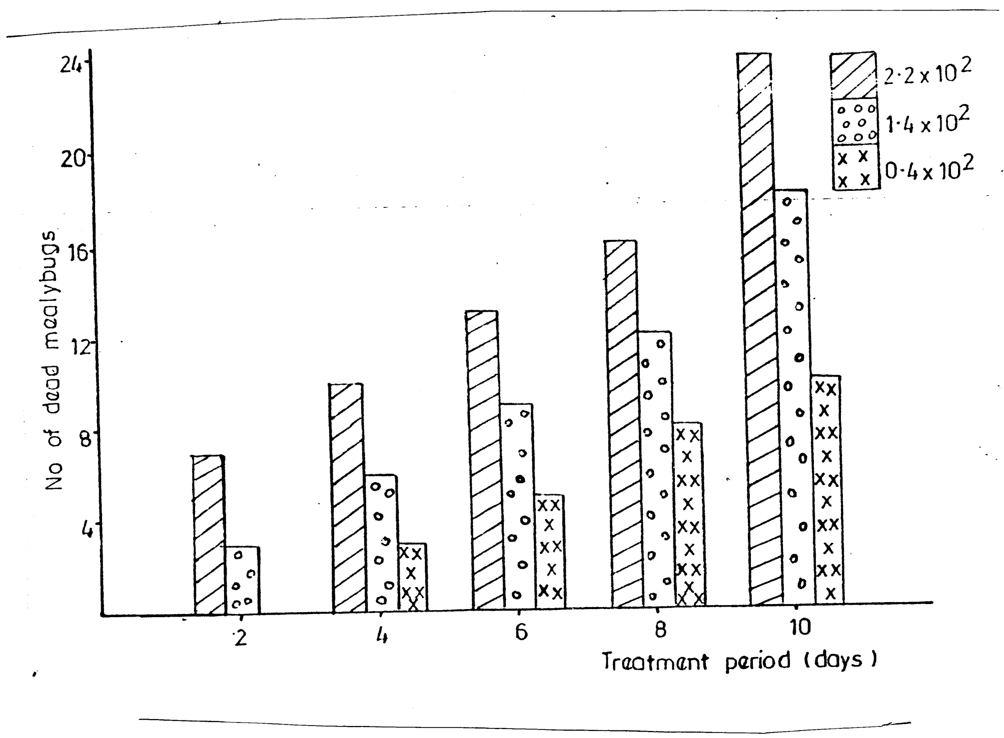

Fig. 3: Effect of different concentrations of *B. thuringiensis* on mortality rate of mealybugs.

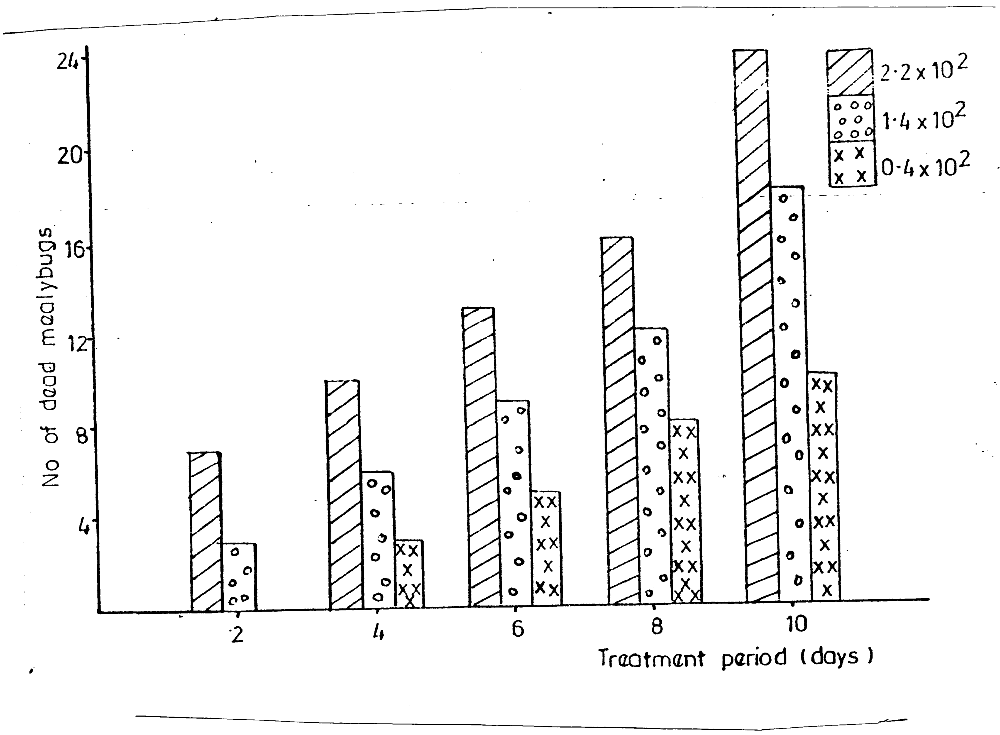

Fig. 3: Effect of different concentrations of *B. thuringiensis* on mortality rate of mealybugs.

Hydrocynus brevis feed mainly on *Alestes* sp. (Reed et al., 1967; Adikwu, 1982) and the presence of prey would determine the presence of predator. Some local extinction like *Heterotis niloticus* in river Yobe at Gashua could result in the decline of fish resources through bad management. Most *Heterotis* and *Alestes* caught are small suggesting that the wetlands are over-fished by the increase of human populations and poor floods from the upstream. This is in agreement with the reports of Matthes (1990) on Hadejia-N'guru wetlands inland waters. Many villages have reported the disappearance of economically important species such as *Heterotis niloticus, Gymnarchus, Channa obscura* and prominent amongst them in "Bana" (*Citharinus citharus*) after which a 0.15 km^2 matural depression Southwest of the village was named (Matthes, 1990).

The fishing methods used in the wetlands are very diverse with a range of gear types being employed to suit the type of water body being fished and the state of the flood. As the flood recedes, water remains in depressions and as these ponds get smaller the fish become concentrated, making them easy to catch before the water dries up completely.

The major conclusion from these studies is that the Hadejia-N'guru wetlands would be of greater value to Nigeria if the present system of fishing is retained and its efficiency is enhanced by the deployment of appropriate technology in the area. Thus, the production from the wetlands could be sustainable in the longer term, provided the flood continues. Since little capital cost to state or national government is incurred in their maintenance, with the attendant practice of maintenance of an area of exceptional biological importance, particularly for wintering plaeartic and breeding Afro-tropical waterfowls (Stove, 1988). This suggests an effort on regulation of Basin water for the availability of water from upstream and fishery management policy by limiting the mesh size to avoid the catch of small fish (SL = 4cm). In comparison with the other parts of northern Nigeria, the Hadejia-N'guru wetlands have higher economic, hydrological, socio-economic and conservation value (Barbier et al., 1991). Their loss would be a net economic and cultural loss for Nigeria.

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References

Aboubakar, J. (1990) Fuelwood and fishery activities in the Hadejia-N'guru wetlands. A Document of the Hadejia-N'guru Wetlands Conservation Project.

Adikwu, I. A. (1982) Some aspects of the biology of *Hydrocynus brevis* (Gunther) in Tiga Lake, Nigeria. M.Sc. Thesis, Department of Biological Sciences, Bayero University, Kano, Nigeria.

Barbier, E. B.; Adams, W. M. and Kimmage, K. (1991) Economic valuation of wetland benefits: The Hadejia-Jama'are floodplain, Nigeria. London Environmental Economics Centre.

Elgood, J. N. (1977) Water resource development in Kano State and its effects on the environment and wildlife, especially fish and aquatic birds.

Jimoh, M. A. (1989) Fuelwood-fishery activities in the Hadejia-N'guru wetlands. A Document of the Hadejia-N'guru Wetlands Conservation Project.

Lagler, K. F. (1970) Capture, sampling and examination of fishes. In: Methods for Assessment of Fish Production in Freshwaters. (W. E. Rickor, ed.). IBP Handbook 3. Blackwell Scientific Publications, Oxford and Edinburgh, pp. 7 - 95.

Leveque, C. D.; Paugy and Teugels, G. G. (Eds.) (1992) Faune des poissons d'eaux douces et saumatres de L'Afrique de l'Ouest (Tome 2) ORSTOM et MRAC Tervuren et Paris. 902pp.

Matthes, H. (1990) Report on the fishery related aspects of the Hadejia-N'guru wetlands Conservation Project. Mission Report. A Document of the Hadejia-N'guru Wetlands Conservation Project.

Reed, W. (1967) The fish and fisheries of Northern Nigeria. Gaskiya Corp., Zaria.

Reed, W. and Holden, M. (1972) West African Freshwater Fish. Longman, Singapore.

Stove, T. J. (1988) Waterbird Survey: Hadejia Wetlands, Nigeria. 10/1 - 7/2/1988. RSPB, United Kingdom.

Thomas, D. (1990) Technical Consultant Preliminary Report, January 1990. A Document of the Hadejia-N'guru Wetlands Conservation Project.

Welcomme, R. L. (1985) River Fisheries. FAO Fisheries Technical Paper 262.