PROTOCOLS FOR THE BIOLOGICAL EVALUATION OF PESTICIDES ON SELECTED CROPS GROWN IN BOTH THE HUMID AND SAHEL REGIONS OF WEST AFRICA

PREPARED UNDER THE AUSPICES OF THE WEST AFRICA AGRICULTURE PRODUCTIVITY PROGRAMME (WAAPP)

ENVIRONMENTAL PROTECTION AGENCY

CHEMICALS CONTROL AND MANAGEMENET CENTRE

ACCRA

JUNE 2012

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CHAPTER 1

FRAMEWORK PROTOCOL FOR BIOLOGICAL EVALUATION OF FUNGICIDES AND BACTERICIDES

Introduction

This Framework Protocol has been prepared to facilitate the conduct of field trials and judicious comparison of test results in all Member States of ECOWAS, CILSS and UEMOA.

This section defines the conduct of biological evaluation of tests of fungicides and bacteriocides for the control of plant pathogens.

Approvals and Amendments

Initial Approval by WAPRC: ..... July 2008 under Framework Protocol No. PC 6

1.0 Experimental Conditions

1.1 Target Pathogens, Selection of Crops and Cultivars

The pathogen to be controlled and the crop to be protected should be clearly specified. For example: *Pyricularia oryzea* that affects rice of both local and improved varieties. The variety or cultivar used should be specified.

The susceptible sensitive cultivar or variety commonly used in the zone should be used for the test.

The product should be applied on the crops and the pathogens according to the manufacturer's recommendations.

1.2 Test Conditions

The test conditions should be favourable for the development of the pathogen. The agronomic practices should be uniform in all the test plots and consistent with Good Agricultural Practices (GAP) under local conditions.

The test should be carried out in areas where the pest pressure is generally high. The cropping condition (soil type, fertilizer application, tillage, cultivar, row spacing) should be uniform in all the test plots and consistent with GAP under local conditions. The cropping history and the pesticide products applied in the preceeding two years must be known.

The test should be conducted in similar or distinct agro-climatic conditions, preferably in different years or growing seasons. The exact number of tests to be conducted will be spelt out in the harmonized document containing pesticides registration requirements in Member States of ECOWAS, CILLS and UEMOA.

1.3 Experimental Procedures and Conduct of Tests

The test should comprise at least three (3) treatments: the product to be tested, the reference product and the untreated control.

The experimental design should be a Randomized Complete Block Design (RCBD) with four replications. Enough space should be provided between the blocks and between the plots. The minimum sizes of the plots depend on the crop.

- Examples of some minimal plot sizes are: Cereals and cotton: 100 m^2

- Grain legume, annual oil seed crops and vegetables: 25 m^2
- Rice: 20 m^2
- Roots and tubers: 50m^2
- Tree crops and oil palm: at least five(5) trees

2.0 Application of Treatment

2.1 Product(s) under study

The product to be tested should be a formulated product and should have a specific name.

2.2 Reference Product

In view of the variability of conditions under which pesticides are used, it is necessary to include a reference product in field trials to allow meaningful evaluation of efficacy under the conditions of the trial. Satisfactory levels of efficacy will generally be met when performance is comparable to that of the reference product, which should preferably be a registered product widely accepted as satisfactory in practice. However, other considerations (e.g. manner of use, side effects, etc may arise in assessing what is a satisfactory level of efficacy.

Where the type of pesticide product or its use is new, comparison with a reference product may be impossible or inappropriate. In this case, the product under study should show a consistent well-defined benefit. The pesticide to be introduced should be able to bring and/or keep the pest population and the damage to which it gives rise below an economic or phyto-sanitary threshold level, where this is known.

Wherever feasible the reference product chosen should be one which has shown satisfactory results in practice and its mode of action should be the same as or similar to that of the test product. The registration authority must be consulted on issues relating to the selection of a reference product for the study. Where a there is the need to use a reference product other than the one agreed or recommended by the registration authority, a justification must be made.

2.3 Untreated Control

An untreated control is needed for on-site tests as well as test conducted under local farming conditions.

2.4 Application Methods

Applications should conform to good agricultural practices.

2.4.1 Method of *Application*

The method of application should be that which is recommended and should be specified. (Example: spraying, vaporization, fumigation, dust application and/or incorporation of granules into the soil).

2.4.2 Type of *Application Equipment*

The type of application equipment used should be specified. In all cases, the application should be done with the aid of equipment that ensures uniform distribution of the product over the entire plot or that which ensures guided on-target treatment. Factors likely to affect the efficiency of delivery (pressure and type of nozzle) should be

selected in accordance with product recommendations. Seeds should be treated with equipment that allows for uniform distribution of the product in accordance with recommended practice.

2.4.3 Timeframe and Frequency of *Application*

The number of applications and the date of each application should be as recommended for the proposed usage. These will depend on the objective of the evaluation and should be consistent with the stage of development of the crop and the target organism.

2.4.4 Application Rates and Volumes

The product should be tested using the recommended rate(R). The rate should be expressed in kg/ha or l/ha of the formulated product. It may also be necessary to express this in g. ai/kg or g .ai/l. A minimum of three rates should be tested on site; that is, the rate recommended by the manufacturer, a lower rate and a higher rate. The exact choice of rates is expected to help determine whether the rate recommended by the manufacturer is the optimal in terms of efficacy and economic returns under any given agro-ecologic condition.

In local farming setting, the optimal rate ascertained from on-site tests is generally tested.

For sprayings, the data regarding their concentration (g.ai/l) should be further specified. The volume per plant should be indicated. For very high vapour pressure products (fumigants, aerosols and vaporizers), the dose administered should be expressed in square meter of surface, and per cubic meter volume of greenhouse.

2.4.5 Information on all other pesticide products

Where other pesticide products (or biological control agents) are used, they should be applied uniformly on all the plots. They should not be mixed with the product under study and the reference product. The exact dates on which such treatments were applied should be recorded. .

3.0 Observations, Data Collection and Measurements

3.1 Meteorological and Edaphic/Soil Data

3.1.1 *Meteorological Data*

The data obtained from the test site or from the nearest meteorological station should be specified. Such data should include precipitations (frequency and volume) and temperature (mean, maximum and minimum in^{o} C). All significant changes in the weather should be noted, particularly those likely to affect the test (hygrometry, dry spells). Precise data on all possible irrigation of the area should be indicated.

3.1.2 Edaphic/Soil Data

During conduct of the test, it will be useful to be aware of the soil pH (10-20 cm) structure and texture (granulometry and organic content).

3.2 Method, Time and Frequency of Observations

3.2. 1 *Method*

Data on seedling emergence vigor, density and on the incidence and severity of infection should be recorded. . The rating scale used in the observation should be specified. The growth stage of crop should be noted during each application.

3.2.2 Timeframe and Frequency of observation

The timeframe and frequency of the observation should be specified: the first

evaluation should be conducted just before the treatment; followed by one or several intermediary evaluations and a final evaluation.

3.3 Observations of Phytotoxicity on Crop

All positive effects of the treatment on the plants should be noted. The crop should be examined for the presence or absence of phytotoxic effects. In case of phytotoxicity, the symptoms (stunting, chlorosis, deformation, etc) should be described.

3.4 Observations of the Effects on Non-Target Organisms

3.4.1 Effects on other pest organisms

All observed effects on other pests including those not targeted, be it positive or negative, should be noted.

3.4.2 Effects on other beneficial organisms

Any positive or negative effects observed on parasitoids and predators, pollinators present naturally or introduced, as well as on adjacent or succeeding crops, should be noted. Any effect on the environment, especially the effects flora and fauna, should be described.

3.5 Quantitative and Qualitative Evaluation of Harvested Crop

The yield and quality of the harvested produce should be assessed. The yield assessment methods and the quality index measurement standards used should be described.

4.0 Results

Test results should be presented in systematic and easily understandable form. They should be subjected to appropriate statistical analysis.. The report will include analysis and interpretation of the relevant data. Other stages of the evaluation will then follow. *Vide* OEPP PP 1/152 (2) standard Directive for the conduct and analysis of biological evaluation tests and OEPP PP 1/181 standard - Directive on biological evaluation of phytosanitary products for the conduct of biological evaluation tests and presentation of reports.

CHAPTER 2

FRAMEWORK PROTOCOL FOR BIOLOGICAL EVALUATION OF HERBICIDES

Introduction

This Framework Protocol has been prepared to facilitate the conduct of experiments and judicious comparison of test results in all Member States of ECOWAS, CILSS and UEMOA.

The objective of this section is to define the general framework for the testing of herbicide products for the purpose of registration. It covers farm weeding and uncultivated areas including land and water.

The following types of assessment should be carried out for any herbicide product.

- Product efficacy on target weeds.
- Product selectivity, residual effect
- Product phytotoxicity
- Crop sensitivity to product
- Product side effect on succeeding crops

Approvals and Amendments

Initial Approval by *WAPRC:* .... July 2008, under Framework Protocol No. PC

1.0 Experimental Conditions

The performance the herbicide should be studied under the different conditions prevalent in Member States of ECOWAS, CILSS and UEMOA in periods of the year when the herbicide is usually used. The tests conducted under different conditions help to express the variability of the performance of the herbicide.

1.1 Target Organisms, Selection of Crops and Cultivars

For evaluation of the efficacy of the product, the experimental plot should have uniform density and varied population of weeds associated with the crop. The vegetation should compose of the target weed specified for the product.

For the purpose of assessing the selectivity of the product, the experimental plot should similarly, and as far as possible, be free of weeds. To eliminate residual weeds, manual, mechanical or chemical weeding should be carried out. However any chemical weed remover used in this connection should not interfere with the product to be tested or with the reference product. Test should be carried out to determine residual effect on succeeding crops.

Varietal sensitivity test are conducted to facilitate the understanding of the selectivity of a product on a crop. Such tests should involve a large number of cultivars in several sites under distinct agro-climatic conditions, with limited number of replications. The test does not entail yield assessment.

The experimental conditions of these two types of test should be identical to those of the selectivity test. The crop and cultivars used should be defined. The cultural practices (agronomic) should be those practiced locally.

1.2 Test Conditions

The test should be carried out in areas where the target weed population is generally high. The tests should be carried out in at least three (3) areas of West Africa in distinct agro-climatic conditions preferably in different years or growing periods. The exact number of tests to be conducted should be spelt out in the document defining the pesticides registration requirements in Member States of ECOWAS, CILLS and UEMOA.

The cropping conditions should be uniform in all the test plots and in accordance with local practice. The cropping history and herbicides used should be recorded. The latter should have no toxic effects on the crop.

1.3 Experimental Procedures and Conduct of Tests

1.3.1 On-site test

On-site evaluation of the biological efficacy of a herbicide may involve the following assessments:

- Efficacy
- Selectivity
- Side effects and
- Varietal sensitivity

Depending on the type of test, the treatment should comprise the product under study, the reference product and untreated control.

The experimental procedures should be consistent with the objectives of the test type.

Ex. 1: the reference treatment is adjacent where each plot treated is contiguous to a non-treated plot for the purpose of efficacy assessment:

Ex.2: randomized block design in case of mono-specific weed invasion for evaluation of efficacy, and:

Ex.3: randomized block for assessment of selectivity.

Plot size will depend on the type of crop and treatment applied. Examples of plot sizes are:

- wide spaced-out crops (forest and fruit tree species): $50m^2$
- medium spaced-out crops(cotton, maize, sorghum and millet):$20m^2$
- closely spaced crops(cassava, yam, cocoyam, rice, cowpea, sweet potato): $15m^2$

The number of replications for each treatment should be at least 4.

1.3.2 Test under local farming conditions

Biological efficacy tests for herbicides under local conditions are generally known as the on-farm evaluation of herbicides. The treatment consists of the product to be tested at a rate defined during on-farm testing, the reference product and the farmer's local practice. The plots are made out in randomized blocks in accordance with the test procedure. At least 10 replications per treatment is proposed. The plot size depends on the type of crop and application equipment. This should be $100m^2$ or more. For treatments where there is risk of interference, steps should be taken to ensure appropriate isolation of the plots.

2.0 Application of Treatments

2.1 Product(s) under study

The product to be tested should be a formulated product and should have a specific name.

2.2 Reference Product

In view of the variability of conditions under which pesticides are used, it is necessary to include a reference product in field trials to allow meaningful evaluation of efficacy under the conditions of the trial. Satisfactory levels of efficacy will generally be met when performance is comparable to that of the reference product, which should preferably be a registered product widely accepted as satisfactory in practice. However, other considerations (e.g. manner of use, side effects, etc may arise in assessing what is a satisfactory level of efficacy.

Where the type of pesticide product or its use is new, comparison with a reference product may be impossible or inappropriate. In this case, the product under study should show a consistent well-defined benefit. The pesticide to be introduced should be able to bring and/or keep the pest population and the damage to which it gives rise below an economic or phyto-sanitary threshold level, where this is known.

Wherever feasible the reference product chosen should be one which has shown satisfactory results in practice and its mode of action should be the same as or similar to that of the test product. The registration authority must be consulted on issues relating to the selection of a reference product for the study. Where a there is the need to use a reference product other than the one agreed or recommended by the registration authority, a justification must be made.

2.3 Untreated Control

An untreated control is needed for on-site tests.

2.4 Application Methods

Applications should conform to Good Agriculture Practice (GAP)

2.4.1 Method of application

The method of application should be that which is recommended and should be specified (foliar spray, incorporation, granular application)

2.4.2. Type of Application Equipment

The product should be adapted to the type of application equipment. It should allow uniform distribution of the product over the targeted areas. The pressure, type of nozzle and depth of incorporation should be chosen in accordance with proposed recommendations.

2.4.3. Application period and frequency

The number of applications and date of each treatment should be as recommended for the proposed usage. These will depend on the objective of the evaluation and should be consistent with stage of development of the crop and weeds. For incorporated treatments, the number, depth, the intervals between the incorporation and the type of equipment used should be recorded.

For pre-seeding treatments, the interval (days) between crop emergence and the application should be as recommended. The stage of development of the crop and the weeds should be specified.

For pre-harvest treatments, the interval between the harvest and application of the product should be as recommended.

2.4.4 Application rates and volumes

The product should be tested using the recommended rate(R). The rate should be expressed in kg/ha or l/ha of the formulated product. It may also be necessary to express this dose in g ai/kg or g.ai/l.

With respect to efficacy tests, at least two (2) supplementary rates should be used, a lower and a higher rate. The exact choice of rate is expected to help determine whether the rate recommended by the manufacturer is the optimal rate in terms of efficacy and economic returns under a given agro-ecological conditions.

For assessment of selectivity, a higher rate (2R) and possibly 3(R) should be included in the treatment.

The volume of water used depends on the rate of application the type of product and the application equipment. This should be specified.

The actual rate applied should always be measured and any deviation from the recommended rate determined.

2.4.5 Information on all other pesticide products

Where other pesticide products (or biological control agents) are used, they should be applied uniformly on all plots. They should not be mixed with the product under study and the reference product. The date on which the treatments were applied should be recorded. The risk of interference should be minimized.

3.0 Observation and Data Collection

3.1 Meteorological and Edaphic Data

The meteorological data recorded on the day of treatment application should include rainfall, characteristics of the precipitation (nature, duration, intensity and volume in mm), temperature (mean, minimum and maximum in ^{o}C) wind (speed and direction), cloud cover, sunlight and relative humidity.

Ten days before and after treatment application, the meteorological data likely to influence crop development and/or that of the target weeds and herbicide action should be recorded. The data should be recorded preferably on the test site, but could be obtained from the meteorological station.

On the day of treatment application, the meteorological data likely to influence the quality and persistency of product should be recorded. Such data will normally include precipitations (nature and volume in mm), temperature (mean, maximum and minimum in ^{o}C). Where there are significant changes in the weather in the day of application, these must be recorded.

3.1.1 Edaphic Data

The edaphic data to be gathered include soil type (international standards to be specified), pH, organic matter content, soil moisture, and soil structure and texture and soil fertility status.

3.2 Method Time and Frequency of Observations

The observation should make it possible to have a clear idea of the effects of the product on all weeds (general efficacy), on each species (specific efficacy) and on the crop (phytotoxicity).

3.2.1 Method of Observation

Observations may be quantitative or qualitative.

3.2.1.1 Observations on weeds

Quantitative methods consist of counting or determination of weed biomass (or particular organs). The weeds should be classified either by group (broadleaf and narrow leaf) or by species.

Qualitative methods are based on visual observations. These methods should be simple, rapid and reproducible and lend themselves to statistical analysis. The currently used method is the linear method on rating scale 0-100, where 0 represents treatment without weeds and 100 stands for the same level of infestation as untreated reference object.

Alternatively, the efficacy of the test product can be determined qualitatively using scale of 0-100, where 100 indicates complete weed control by product and 0, is no control.

3.2.1.2 Observations on crops

Phytotoxicity assessment may be carried out in absolute manner where the effects are measurable (example presence of dead crops or injured plants.) in other cases, this can be done qualitatively by estimating phytotoxicity percentage in relation to the untreated reference plot. In all cases phytotoxicity symptoms (stunting, chlorosis, deformation, necrosis etc) should be described. Any effects on subsequent crops should also be recorded.

3.2.2 Timeframe and frequency of observation

Timeframe and frequency of observation are specific to each crop. They should be defined in specific protocols.

3.3 Observations of the Effects on Non-Target Organisms

All observed effects on other organisms either positive or negative should be recorded.

3.4 Quantitative and Qualitative Evaluation of Harvested Crops

Selectivity assessment tests should be conducted. Crop harvesting is optional for efficacy tests. Crops harvesting will be defined in specific protocols.

4.0 Results

Test results should be presented in systematic and easily understandable form. They should be subjected to statistical analysis using pre-established methods. The report should include analysis and interpretation of the relevant data. Other stages of the evaluation will then follow. *Vide* OEPP PP 1/152 (2) standard - . Directive for the conduct and analysis of biological evaluation tests and OEPP PP 1/181 standard - Directive on biological evaluation of phytosanitary products for the conduct of biological evaluation tests and presentation of reports.

CHAPTER 3

FRAMEWORK PROTOCOL FOR BIOLOGICAL EVALUATION OF INSECTICIDES AND ACARICIDES

Introduction

This Framework Protocol has been prepared to facilitate the conduct of field trials and effective comparison of test results in all Member States of ECOWAS, CILSS and UEMOA.

This section defines the general principles for biological evaluation of new active ingredients or formulations of insecticides and acaricides.

Approvals and Amendments

Initial Approval by WAPRC: ..... July 2008 under Framework Protocol No. PC 2

1.0 Experimental Conditions

1.1 Target Pest, Selection of Crops and Cultivars

The pest(s) species as well as the crop varieties should be specified. For example *Coniesta ignefusalis* (millet stalk borer) on *Pennisetum tyhoides* (millet)

The test should be conducted at peak period and in relation with the biology and population dynamics of the species. The test should be carried out on the target pest(s), and their stages of development as recommended.

The crop variety should be susceptible to the pest to be controlled. Where seeds are to be treated, the rate of germination should be assessed.

1.2 Test Conditions

The test should be carried out in areas where the pest pressure is generally high. The cropping condition (type of soil, fertilizer application, tillage, cultivar, row spacing) should be uniform in all the test plots and consistent with good agriculture practices. The cropping history and pesticide products applied in the preceding two years must be known.

The test should be conducted in similar or distinct agro-climatic conditions preferably in different years or growing seasons. The exact number of test to be conducted will be spelt out in the harmonized document containing pesticides registration requirements in Member States of ECOWAS, CILLS and UEMOA.

1.3 Experimental Procedures and Conduct of Tests

The experimental procedure for the test will depend on the crop, pest species, method of treatment and the application equipment used.

1.3.1 On-site test

Treatments: these consist of the product under study, a reference product and an untreated control. The plots are laid out in randomized complete block design. The plot size is generally 25-300m^2. At least 4 replications per treatment are required.

The plots should be arranged in a way to avoid cross contamination of treatments. This can be done by providing sufficient space between the plots and taking into consideration the wind direction during treatment or by using protective screens between the plots.

1.3.2 Test in local farming condition

Treatments: these are the product under study, the reference product and untreated control. The plot should be in randomized complete block design. The plot size is generally 100-5000m^2 owing to the wide range of variations between the plots and

the possibility of losing some of the treatments; at least 5 replications are required for every treatment.

2.0 Application of Treatment

2.1 Product(s) Under Study

The product to be tested should be a formulated product and should have a specific name.

2.2 Reference Product

In view of the variability of conditions under which pesticides are used, it is necessary to include a reference product in field trials to allow meaningful evaluation of efficacy under the conditions of the trial. Satisfactory levels of efficacy will generally be met when performance is comparable to that of the reference product, which should preferably be a registered product widely accepted as satisfactory in practice. However, other considerations (e.g. manner of use, side effects, etc may arise in assessing what is a satisfactory level of efficacy.

Where the type of pesticide product or its use is new, comparison with a reference product may be impossible or inappropriate. In this case, the product under study should show a consistent well-defined benefit. The pesticide to be introduced should be able to bring and/or keep the pest population and the damage to which it gives rise below an economic or phytosanitary threshold level, where this is known.

Wherever feasible the reference product chosen should be one which has shown satisfactory results in practice and its mode of action should be the same as or similar to that of the test product. The registration authority must be consulted on issues relating to the selection of a reference product for the study. Where a there is the need to use a reference product other than the one agreed or recommended by the registration authority, a justification must be made.

2.3 Untreated Control

An untreated control is needed for on-site tests. Test should be conducted under local farming conditions.

2.4 Application Methods

Application should conform to good agriculture practice.

2.4.1 Method of application

The recommended method of application should be used.

2.4.2 Type of application equipment

Each application should be done with the aid of equipment that ensures uniform distribution of the product over the entire plot or that ensures guided target treatment. Factors likely to efficiency of delivery such as pressure or nozzle type should be avoided. The equipment should be selected in accordance with product recommendation.

Seeds should be treated with equipment that allows for uniform distribution of the product in accordance with recommended practice.

2.4.3 Timeframe and frequency of application

The number of applications and date of each treatment should be as those indicated for the proposed usage. These will depend on the biology of the target pest. The date of the

applications should be recorded.

2.4.4 Application rate and volumes

The product should be tested using the recommended rate(R). With respect to efficacy tests, at least two (2) supplementary rates should be used, a lower and a higher rate. The exact choice of rate is expected to help determine whether the rate recommended by the manufacturer is the optimal rate in terms of efficacy and economic returns under a given agro-ecological conditions.

In on-farm setting, the optimal rate determined from on-site test is generally tested.

The rate should be expressed in kg/ha or l/ha of the formulated product and in g ai/h for the active ingredient. It may also be necessary to express this dose in g ai/kg or g.ai/l. for formulations in powder for dust spraying, be it granulated or similar products; this should be expressed in g ai/kg or %.

For seed treatment, the rate should be expressed in kg (or litres) of the formulated product per ton of seeds, and in g ai/kg of seeds.

The actual rate applied should always be measured and any deviation from the recommended rate recorded.

2.4.5 Information on other pesticide products

Where other pesticide products (or biological control agents) are used, they should be applied uniformly on all plots. They should not be mixed with the product under study and the reference product. The date on which the treatments were applied should be recorded. The risk of interference should be minimized.

3. 0 Observations and Data Collection, Recordings And Measurements

3.1 Meteorological and Edaphic Data

Days before and after product application, the meteorological data likely to influence crop development and/or that of the target pest and the action of the pesticide product should be recorded. Such data should normally include rainfall and temperature. All data should in principle, be recorded on the test site, especially for on-site test. It sometimes difficult to obtain meteorological data in the local on-farm condition in which case, the relevant data could be obtained from the nearest meteorological station.

On the day of application, the meteorological data likely to influence the quality and persistency of the product should be recorded. Such data will normally include at least rainfall (nature and volume in mm) and temperature (mean maximum and minimum in ^{o}C) any significant change in the days weather should be recorded and in relation to the application indicated.

Throughout the test period, prolong dry periods, heavy rains, sandy winds etc, likely to influence the outcome of the test should be recorded. Accurate data should be provided on any irrigation to be carried out on the plot.

3.1.1 Edaphic data

The edaphic data to be gathered include soil type (international standards to be specified), pH, organic matter content, soil moisture, and soil structure and texture and soil fertility status.

Where experiments are carried out on plants cultivated in humus or any other artificial media these should be described in detail. The description should include the irrigation system in which the substrates are kept.

3.2 Method, Time and Frequency of Observation

The growth stage of the crop should be recorded during each application.

3.2.1 Method

The sampling or observation method depends on the pest under consideration. The method chosen should be such that statistical analysis can be conducted to assess the efficacy of the product. Details of the method will be defined in the specific protocols for crop/pest formulations.

3.2.2 Timeframe and frequency

At least one observation on the pest is required (generally 1-3 days) before treatment. A sample of pest could be taken before treatment in order to determine the composition of the pest complex or to estimate the degree of insect or mites.

The timeframes and frequencies of observations should also take into account the phonological state of the crop, the level of pest population, the stage of development and the mode of action of product and product persistency.

3.3 Observations of Phytotoxicity

Phytotoxicity should be examined and all positive or negative effects recorded.

3.4 Observations of Effects on Non-Target Organism

3.4.1 Effects on other pest

All observed effects on other pest be it positive or negative should be recorded.

3.4.2 Effects on beneficial organisms

Any positive or negative effect observed on parasitoids and predators, pollinators on adjacent or succeeding crops should be recorded. Any effect on the environment especially the effects on other fauna (both terrestrial and aquatic) should be described.

3.5 Quantitative and Qualitative Evaluation of Harvested Crop

Although it is recognized that crop yield depends on several factors, quantitative and/or qualitative assessment of harvested crop is required. Depending on the crop, such evaluation could include yield assessment (adjusted to humidity rate) the weight of 1000 grains, classification of fruit or legume quality, taint, residue and physical appearance in accordance with national standards.

4.0 Results

Test results should be presented in systematic and easily understandable form. They should be subjected to statistical analysis using pre-established methods. The report will include analysis and interpretation of the relevant data. Other stages of the evaluation will then follow. *Vide* OEPP PP 1/152 (2) standard Directive for the conduct and analysis of biological evaluation tests and OEPP PP 1/181 standard - Directive on biological evaluation of phytosanitary products for the conduct of biological evaluation tests and presentation of reports.

CHAPTER 4

PROTOCOL FOR BIOLOGICAL EVALUATION OF FUNGICIDES TO CONTROL BLACK SIGATOKA DISEASE OF BANANA AND PLANTAIN

Introduction

This framework protocol has been prepared to facilitate the conduct of field trials and judicious comparison of test results in all member states of ECOWAS, CILLS and UEMOA.

It describes the principles for the conduct of biological evaluation tests on new active ingredients or fungicide formulations designed to control black leaf streak (also known as black Sigatoka) disease of banana/plantain.

Approvals and Amendments

Initial Approval by WARPC: ……2009, reference No.….....ECOWAS

1.0 Experimental Conditions

1.1 Target Pathogens, Selection of Crops and Varieties

The ascomycete fungus, *Mycosphaerella fijiensis* Morelet [anamorph: *Paracercospora fijiensis* (Morelet) Deighton] is the causal organism of black sigatoka on both local and improved varieties of plantain. Yellow sigatoka (*M. musicola*) affects mostly banana.

- The varieties or cultivar used should be specified.
- The susceptible cultivar or variety commonly grown in the zone should be used for the test.
- The test should be conducted at the periods when crops are likely to be subjected to significant pathogen infection.
- The product should be applied on the test plants.

1.2 Test Conditions

- The test conditions should be favourable for the development of the pathogen.
- The test should be carried out in areas where there is sufficient presence of the pathogen.
- The agronomic practices should be uniform in all the test plots and consistent with Good Agricultural Practices (GAP) under local conditions.
- The cropping condition (soil type, fertilizer application, tillage, cultivar, row spacing) should be uniform in all the test plots and consistent with GAP under local conditions.
- The cropping history and the pesticide products applied in the preceding two years must be known.

- The test should be conducted in similar or distinct agro-climatic conditions, preferably in different years or growing seasons.
- The exact number of tests to be conducted will be as spelt out in the harmonized document containing pesticides registration requirements in Member States of ECOWAS, CILLS and UEMOA.

1.3 Experimental Procedures and Conduct of Tests

- The test should comprise three (3) treatments: the product to be tested, the reference product and the untreated control.
- The experimental design should be Randomized Complete Block Design (RCBD) with four replications. Each plot should be surrounded by a row of susceptible border plants. The planting materials (one per hole) may be culture plantlets or suckers of similar size and age.
- There should be at least six plants per plot at the recommended spacing metreAbout 5m of space should be provided between the blocks and between the plots.
- It is not always easy to differentiate between the symptoms of the various *Mycosphaerella* leaf spot diseases (Black sigatoka (*M. fijiensis*) and Yellow sigatoka (*M. musicola*)). Where possible, it is preferable to choose sites where only black or yellow leaf spot disease is present. Having a mix of pathogens makes it difficult to compare the results with those from other evaluation sites.

2.0 Application of Treatment

2.1 Product(s) Under Study

- The product to be tested should be a formulated product and should have a specific name, the Material Safety Data Sheet, technical leaflet and draft label.
- In view of the variability of conditions under which pesticides are used, it is necessary to include a reference product in field trials to allow meaningful evaluation of efficacy under the conditions of the trial.
- Satisfactory levels of efficacy will generally be met when performance is comparable to that of the reference product which should preferably be a registered product widely accepted as satisfactory in practice. However, other considerations (e.g. manner of use, side effects, etc) may arise in assessing what is a satisfactory level of efficacy.
- Where the type of pesticide or its use is new, comparison with a reference product may be impossible or inappropriate. In this case, the product under study should show a consistent well-defined benefit.
- The test product should effectively reduce the pest population and the damage below an economic or phytosanitary threshold level, where this is known.

- Where feasible the reference product chosen should be one which has shown satisfactory results in practice and its mode of action should be the same as or similar to that of the test product. The registration authority must be consulted on issues relating to the selection of a reference product for the study. Where there is the need to use a reference product other than the one agreed or recommended by the registration authority, a justification must be made. Registration authorities must assist test Agents to secure appropriate reference products if it becomes necessary.

2.2 Untreated Control

An untreated control is needed for all tests conducted.

2.3 Application Methods

Application of the test and reference products should conform to GAP.

2.3.1 Method of Application

- The recommended method of application should be used

2.3.2 Type of application equipment

- The type of application equipment should be specified. In all cases, the application should be done with the aid of equipment that ensures uniform distribution of the product over the entire plot or that which ensures guided on-target treatment.
- Factors likely to affect the efficiency of delivery (pressure and type of nozzle) should be selected in accordance with product recommendations.
- Planting materials should be treated with equipment that allows for uniform distribution of the product in accordance with recommended practice.

2.3.3 Time and Frequency of Application

The time, interval and number of applications should be as recommended for the proposed usage. These will depend on the objective of the evaluation and should be consistent with the stage of development of the crop and the target organism.

2.3.4 Application Rates and Volumes

- The product should be tested using the recommended rate(R). The rate should be expressed in kg/ha or l/ha of the formulated product. It may also be necessary to express this in g a.i/kg or g a.i/l.
- A minimum of three rates should be tested on-site; that is, the rate recommended by the manufacturer, a lower rate and a higher rate.
- The exact choice of rate is expected to help determine whether the rate recommended by the manufacturer is the optimal in terms of efficacy and economic returns under any given agro-ecological condition.

- In local farming settings, the optimal rate ascertained from on-site tests should be tested.
- The volume sprayed per plant should be indicated. The dose administered for very high vapour pressure products (fumigants, aerosols and vapourizers), should be expressed in per square metre of surface area, and per cubic metre of volume eg in a greenhouse.

2.3.5 Information on all other pesticide products

Where other pesticide products (or biological control agents) are used

- They should be applied uniformly on all the plots.
- They should not be mixed with the product under study and the reference product.
- The exact time, interval and number of applications should be recorded.

3.0 Observations, Data Collection and Measurements

3.1 Meteorological Data

The data obtained from the test site or from the nearest meteorological station should be recorded. Such data should include rainfall/precipitation (frequency and volume) and temperature (mean, maximum and minimum in ^{O}C). All significant changes in the weather should be noted, particularly those likely to affect the test (hygrometry, dry spells). Precise data on all possible irrigation of the area should be indicated.

3.2 Edaphic/Soil Data

The following may be recorded

- Soil pH (10-20 cm)
- Soil structure and texture (granulometry and organic content).

3.3 Method, Time and Frequency of Observations

3.3.1 Method

- Data on disease incidence and severity of infection should be recorded.
- The rating scale used in the observation should be specified (preferably Youngest Leaf Spotted (YLS) and Gauhl's modification of Stover's severity scoring system.
- The growth stage of the crop should be noted during each application.

i) Young leaf spotted (YLS) method (Stover, R.H. 1980. Sigatoka leaf spot diseases of

bananas and plantains. Plant Disease 64:750-756).

- Counting down from the top of the plant, the youngest leaf spotted (YLS) is the leaf number of the first fully expanded leaf with at least 10 discrete, mature, necrotic lesions or one large necrotic area with 10 light-coloured dry centres. (YLS may be recorded from three months after planting).

ii) **Gauhl's modification of Stover's severity scoring system** (Gauhl, F. 1994. Epidemiology and ecology of black Sigatoka (*Mycosphaerella fijiensis* Morlet) on plantain and banana (*Musa* spp.) in Costa Rica, Central America. INIBAP, Montpellier, France. 120pp).

The proportion of the leaf area showing symptoms is scored on a scale of 0 to 6 as follows:

0 = no disease symptom

1 = <1% showing symptoms

2 = 1-5% showing symptoms

3 = 6-15% showing symptoms

4 = 16-33% showing symptoms

5 = 34-50% showing symptoms

6 = >50% showing symptoms

A disease severity index (DSI) is calculated as follows: $\frac{\Sigma nb \times 100}{[(N-1)\ T]}$

Where n = number of leaves in each grade

b = grade

N = number of grades used in the scale (7)

T = total number of leaves graded on each plant

(Disease severity may be recorded from six months after planting)

3.3.2 Timeframe and Frequency of observation

- The timeframe and frequency of observation should be recorded.
- The first observation should be just before the treatment, followed by one or several intermediary and a final observation.

3.4 Observations of Phytotoxicity on Crop

- All effects of the treatment on the plant should be noted.
- The crop should be examined for phytotoxic effects and the symptoms (stunting, chlorosis, deformation, etc) described.

3.5 Observations of the effects on Non-Target Organisms

3.5.1 Effects on other pest organisms

- Effects on other pests including those not targeted, be it positive or negative, should be noted.

3.5.2 Effects on other beneficial organisms

- Effects on parasitoids and predators, pollinators present naturally or introduced as well as on adjacent or succeeding crops, should be noted.

- Effects on the environment, especially t on flora and fauna, should be described.

3.6 Quantitative and Qualitative Evaluation of Harvested Crop

- The yield and quality of the harvested produce should be assessed.
- The yield assessment method and quality index measurement standards used should be described.

4.0 Results

The results should be presented in systematic and easily understandable form and should be subjected to statistical analysis using standard methods. The analyzed data should be interpreted and appropriate recommendations made. (*Vide* OEPP PP 1/152 (2) Standard Directive for the conduct and analysis of biological evaluation tests and OEPP PP 1/181 standard –Directive on biological evaluation of phytosanitary products for the conduct of biological evaluation tests and presentation of reports).

CHAPTER 5

PROTOCOL FOR BIOLOGICAL EVALUATION OF FUNGICIDES TO CONTROL MANGO ANTHRACNOSE

Introduction

This framework protocol has been prepared to facilitate the conduct of field trials and judicious comparison of test results in all member states of ECOWAS, CILLS and UEMOA.

It describes the principles for the conduct of biological evaluation tests on new active ingredients or fungicide formulations designed to control mango anthracnose.

Approvals and Amendments

Initial Approval by WARPC: ……2009, reference No.….....ECOWAS

1.0 Experimental Conditions

1.1 Target Pathogens, Selection of Crops and Cultivars/varieties

The pathogens to be controlled, Colletotrichum *gloeosporioides* and *Botryodiplodia theobromae. Colletotrichum gloeosporioides* are known worldwide as the causal organism for mango anthracnose disease. *Botryodiplodia theobromae*, however, has been isolated frequently alongside. *C. gloeosporiodes* from brown to black lesions on ripened mango fruits (characteristic symptom of mango anthracnose) collected from all agro-ecologies where mango is grown. In relatively very moist agro-ecologies, *B. theobromae* may be frequently isolated from fruit lesions more than *C. gloeosporioides*. This implicates *B. theobromae* in fruit rot diseases.. Any effective disease control strategy against anthracnose to improve yield and fruit quality must therefore, target the two pathogens.

Several of the introduced and local varieties of mango are susceptible to anthracnose disease. ‘Haden’ ‘Keitth’ and ‘Irwin’ are very susceptible to anthracnose and testing for fungicide efficacy against anthracnose using a field of any of these two varieties is recommended if they are available.

In the absence of a very susceptible variety, a susceptible cultivar with a recognized economic importance in the test zone should be selected for the test.

1.2 Test Conditions

The test should be carried on mango fields in a relatively humid environment where favourable conditions for *C. gloeosporioides* and *B. theobromae* exist during flowering. Anthracnose thrives well under favourable climatic conditions of high relative humidity, frequent rains and a temperature of 24◦ to 32◦ C. Susceptible cultivars/varieties growing under these conditions will be suitable for testing for fungicide efficacy against anthracnose disease.

Inoculum levels of both pathogens may not be sufficiently high to present a high disease pressure necessary to challenge the potency of the test product if a dry zone with a low relative humidity is selected as the test location.

A fungicide that controls mango anthracnose in a higher rainfall and high relative humidity zone will do even better against the disease in a drier environment.

In addition to the above, the test should be carried out in an orchard with a reported history of mango anthracnose to ensure a higher disease pressure.

The cropping condition (e.g. soil type, fertilizer application, tillage, cultivar, row spacing) should be uniform in all the plots and should be consistent with good Agricultural Practices (GAP) under local conditions.

Spacing between plants should be as recommended for the cultivar/variety selected for the study.

The cropping history and the pesticides applied in the preceding two years before the test must be known.

The test must be conducted in similar or distinct agro-climatic conditions for a period not less than two years but preferably three years. Where two seasons of fruit harvest exist in a year, tests should be carried in both seasons to establish the performance of the product in each season.

1.3 Experimental Procedures and Conduct of Tests

The test should comprise three (3) treatments: the product to be tested, the reference product and the untreated control.

Treatments should be arranged in a Randomized Complete Block Design (RCBD) with four replications. Enough space (5-10m, depending on cultivar/variety), should be provided between the blocks and between the plots.

- Each plot should have at least 6 mango trees that have reached fruit bearing stage.
- Selected trees should be uniform and of the same age.

2.0 Application of Treatment

- The product to be tested should be a formulated product and should have a specific name, the Material Safety Data Sheet, technical leaflet and draft label.
- In view of the variability of conditions under which pesticides are used, it is necessary to include a reference product in field trials to allow meaningful evaluation of efficacy under the conditions of the trial.
- Satisfactory levels of efficacy will generally be met when performance is comparable to that of the reference product which should preferably be a registered product widely accepted as satisfactory in practice. However, other considerations (e.g. manner of use, side effects, etc) may arise in assessing what is a satisfactory level of efficacy.

- Where the type of pesticide or its use is new, comparison with a reference product may be impossible or inappropriate. In this case, the product under study should show a consistent well-defined benefit.
- The test product should effectively reduce the pest population and the damage below an economic or phytosanitary threshold level, where this is known.

Where feasible the reference product chosen should be one which has shown satisfactory results in practice and its mode of action should be the same as or similar to that of the test product. The registration authority must be consulted on issues relating to the selection of a reference product for the study. Where there is the need to use a reference product other than the one agreed or recommended by the registration authority, a justification must be made. Registration authorities must assist test Agents to secure appropriate reference products if it becomes necessary.

2.1 Untreated Control

An untreated control is needed for all tests conducted.

2.2 Application Methods

Application of the test and reference products should conform to GAP.

2.2.1 Method of Application

- The recommended method of application should be used

2.2.2 Type of application equipment

- The type of application equipment should be specified. In all cases, the application should be done with the aid of equipment that ensures uniform distribution of the product over the entire plot or that which ensures guided on-target treatment.
- Factors likely to affect the efficiency of delivery (pressure and type of nozzle) should be selected in accordance with product recommendations.
- Planting materials should be treated with equipment that allows for uniform distribution of the product in accordance with recommended practice.

2.2.3 Time and Frequency of Application

The time, interval and number of applications should be as recommended for the proposed usage. These will depend on the objective of the evaluation and should be consistent with the stage of development of the crop and the target organism.

2.2.4 Application Rates and Volumes

- The product should be tested using the recommended rate(R).
- The rate should be expressed in kg/ha or l/ha of the formulated product.
- It may also be necessary to express this in g a.i/kg or g a.i/l.

- A minimum of three rates should be tested on-site; that is, the rate recommended by the manufacturer, a lower rate and a higher rate.
- The exact choice of rate is expected to help determine whether the rate recommended by the manufacturer is the optimal in terms of efficacy and economic returns under any given agro-ecological condition.
- In local farming settings, the optimal rate ascertained from on-site tests should be tested.
- . The volume sprayed per plant should be indicated. The dose administered for very high vapour pressure products (fumigants, aerosols and vapourizers), should be expressed in per square metre of surface area, and per cubic metre of volume eg in a greenhouse.

2.2.5 Information on all other pesticide products

Where other pesticide products (or biological control agents) are used

- They should be applied uniformly on all the plots.
- They should not be mixed with the product under study and the reference product.
- The exact time, interval and number of applications should be recorded.

3.0 Observations, Data collection and Measurements

3.1 Meteorological Data

- The data obtained from the test site or from the nearest meteorological station should be recorded. Such data should include rainfall/precipitation (frequency and volume) and temperature (mean, maximum and minimum in ^{O}C).
- All significant changes in the weather should be noted, particularly those likely to affect the test (hygrometry, dry spells).
- Precise data on all possible irrigation of the area should be indicated.

3.2 Edaphic/Soil Data

The following may be recorded

- Soil pH (10-20 cm)
- Soil structure and texture (granulometry and organic content).

3.3 Method, Time and Frequency of Observations

3.3.1 Method

Incidence and severity of anthracnose disease on panicles, leaves and fruits should be recorded for each tree in a plot just before the first treatment is applied. Further records should be taken at monthly intervals throughout the season and the last record taken two months after harvest.

It is recommended that incidence and severity of anthracnose on trees outside the plots during the period of the study should be recorded.

At harvest, it is recommended that at least 30 matured fruits selected randomly from each plot are incubated at ambient temperature for 16 days to allow the postharvest phase of the disease to be assessed on fruits.

Disease severity assessment of leaves and fruits should be based on a 1 - 5 scale (1 = no visible symptom of disease observed; 2 = a single lesion to few lesions covering 25% of total surface area; 3 = up to 50% of surface area covered by lesions; 4 = up to 75% of surface area covered by lesions; 5 = > 75% of surface area covered by lesions.

3.3.2 Time and Frequency of Observation

- The first observation or assessment should be done before pruning.
- The second assessment should be carried out just before the first treatment.
- Subsequent observations should be carried out at monthly intervals and the final at two months after harvest of fruits**.**

3.4 Observations of Phytotoxicity on Crop

The effects of the treatment on the quality of leaves, branches and fruits must be noted. Phytotoxic effects of the test product (fungicide) such as scorching or 'burns', wilting or bleaching of leaves or developing fruits should be noted.

3.5 Observations of the effects on Non-Target Organisms

3.5.1 Effects on other pest organisms

- Effects on other pests including those not targeted, be it positive or negative, should be noted.

3.5.2 Effects on other beneficial organisms

- Effects on parasitoids and predators, pollinators present naturally or introduced as well as on adjacent or succeeding crops, should be noted.
- Effects on the environment, especially on flora and fauna, should be described.

3.6 Quantitative and Qualitative Evaluation of Harvested Crop

- The yield and quality of the harvested produce should be assessed.
- The yield assessment method and quality index measurement standards used should be described.

3.7 Quantitative and Qualitative Evaluation of Harvested Crop

- The yield of fruits from treated and untreated plots must be recorded and compared statistically to indicate the percent increase or decrease in yield due to the application of the subject product.
- The quality of harvested fruits from treated and untreated plots must be assessed at harvest and during ripening using acceptable quality index measurement standards (the specific index used in assessing fruit quality should be specified). The quality of fruits at full ripening should be recorded.

4.0 Results

The results should be presented in systematic and easily understandable form and should be subjected to statistical analysis using standard methods. The analyzed data should be interpreted and appropriate recommendations made.

(*Vide* OEPP PP 1/152 (2) Standard Directive for the conduct and analysis of biological evaluation tests and OEPP PP 1/181 standard –Directive on biological evaluation of phytosanitary products for the conduct of biological evaluation tests and presentation of reports).

CHAPTER 6

PROTOCOL FOR BIOLOGICAL EVALUATION OF INSECTICIDES AGAINST COCOA (THEOBROMA CACAO) PESTS

Introduction

This framework protocol has been prepared to facilitate the conduct of field trials and effective conduct of biological evaluation tests on major cocoa insect pests in all member states of ECOWAS, CILLS and UEMOA

It describes the principles for the conduct of biological evaluation or testing of insecticide formulations designed to control major cocoa pests (mirids, stink bugs, stem borers, cocoa mealybugs and defoliators)

1.0 Experimental Conditions

1.1 Target Pest, Selection of Crops and Cultivars/varieties

- The trial must be carried out in laboratories, semi field (cage) conditions and well established cocoa farms.
- The trial must be conducted at the period of high pest pressure in relation to the biology and population dynamics of the species.
- The cultivar/varieties may be *Amelonado, Amazonia* or the mixed hybrids of *Theobroma cacao* L.
- The trial should be carried out on the target pest(s), and their stages of development as recommended. The important pests of cocoa are:

- Cocoa mirids: *Sahlbergella singularis and Distantiella theobroma*
- Stink bug: *Bathycoelia thalassina*
- Stem borers: *Eulophonotus myrmeleon*
- Cocoa mealybugs: *Planococcoides njalensis* and *Planococcus citri*
- Defoliators*: Anomis sp and Earias sp*
- Termites

1.2 Conditions for Trials

- The test should be carried out in selected cocoa farms where the target pest populations are generally high.
- The selected area should be uniform (cropping conditions such as soil type, fertilizer application, agronomic practices, variety etc) should be uniform in all the test plots and consistent with Good Agricultural Practices (GAP).
- The pesticide products applied in the preceding two years must be known.
- The test should be carried out in similar or distinct agro climatic conditions of cocoa production zone preferably in different years or growing seasons.

1.3 Experimental Procedures and Conduct of Tests

1.3.1 Application of treatments

- **P**roduct(s) for testing should be a formulated product and should have a name.
- The product(s) should also have material safety data sheets (MSDS).
- In view of the variable conditions under which product(s) will be screened it is important to include a reference product or a standard with known bio-efficacy level to allow meaningful evaluation under the conditions of the trial.
- The experimental procedure for test will depend on the pest species, method of treatment and the application equipment used. However, mirids remain the most important insect pest of cocoa and most insecticide screening is often directed at this pest.
- To The trials should be conducted in the laboratory and in cages (semi field conditions) to determine minimum effective dosage of the insecticide product and at least 2 years on-farm small scale (0.8ha) researcher managed trial.
- Small scale on-farm researcher managed trial should be followed by at least one year large scale (2 - 3ha) researcher and farmer managed trials.
- Residue assessment trials should be done to conclude the test(s).

1.3.2 Laboratory and cage experiments

Laboratory bioassays should be carried out to determine their minimum effective dosages against target pest, followed by cage spray experiments to confirm their effectiveness.

- During laboratory screening, at least 3 rates should be tested, the manufacturer's rate, a lower rate and a higher rate. This exact choice of rate is to help determine if the rate proposed by the manufacturer is the optimal rate in terms of bio-efficacy and economic returns.
- A reference product (registered) with known efficacy against the pest may be used and distilled water as control.
- At the cage test, two rates from the laboratory screening, which causes more than 95% mortality of the target pests, should be used. The cage test should be replicated at least 5 times.

1.3.3 First year small-scale, researcher-managed field trials

- The minimum effective dosages obtained from the cage test should be tested on-farm in small scale researcher managed trials including a reference product of known efficacy for comparison.
- For each product, 25-50 sites should be selected to serve as replicates and at each site, the plot size should be at least 0.4ha.
- The insecticide to be tested should be applied 2 – 4 times per season depending on the product, using recommended application equipment.

1.3.3.1 Untreated control.

Due to unacceptable damage to untreated control plots, a reference or standard product is used for comparison with the test product.

1.3.3.2 Application Rates and Volumes

- The product should be tested using the recommended rate(R).
- The rate should be expressed in kg/ha or l/ha of the formulated product.
- It may also be necessary to express this in g a.i/kg or g a.i/l.
- A minimum of three rates should be tested on-site; that is, the rate recommended by the manufacturer, a lower rate and a higher rate.
- The exact choice of rate is expected to help determine whether the rate recommended by the manufacturer is the optimal in terms of efficacy and economic returns under any given agro-ecological condition.

1.3.3.3 Method of application

This should conform to GAP. Recommended pneumatic with the right nozzle type or motorized sprayers which ensure uniform distribution of the product over the entire plot should be used.

1.3.3.4 Observations and data collection, recordings and measurements

- Number of target pests from the ground to hand-height (about 2m) on all cocoa trees in each plot should be assessed prior to application of the insecticides and repeated 48 hours thereafter.
- The numbers of the target pest should be recorded every month.

2.0 Observations of the effects on Non-Target Organisms

2.1 Number of Trees with Beneficial Organisms

- Number of cocoa trees harbouring non-target fauna, including predatory insects and spiders should be recorded.

3.0 **Effect on Environment**

- Effects on the environment, especially on flora should be described.

3.1 Meteorological Data

- The data obtained from the test site or from the nearest meteorological station should be recorded. Such data should include rainfall/precipitation (frequency and volume) and temperature (mean, maximum and minimum in ^{O}C).
- All significant changes in the weather should be noted, particularly those likely to affect the test (hygrometry, dry spells).

3.2 Edaphic/Soil Data

The following may be recorded

- Soil pH (10-20 cm)

- Soil structure and texture (granulometry and organic content).

4.0 Second Year Small-Scale, Researcher-Managed Field Trials

- Products that showed promising results in the first year must be tested again in the second year using the same procedures.
- However, if the product(s) perform consistently, the 2nd year small scale field trials can be run parallel with the first year large scale screening.

  The same procedures, observations and data collection as in the first year must be used.

4.1 First Year Large-Scale, Researcher/Farmer-Managed Trial

- Based on the results of the first and second year small scale-farmer managed trials, the **p**roducts should be applied at the previously determined dosage in the first year large scale trial.
- Twenty-five to fifty farmers from all cocoa production zones of the country should be involved with the trial
- Each farmer should be given sufficient quantities of the test and reference products to cover about 2ha (5 acres) farm size.
- Farmers should be taught how to apply the product and advised to observe safety measures.

4.1.1 Untreated control

Due to unacceptable damage to untreated control plots, a reference or standard product is used for comparison with the test product.

4.1.2 Method of application.

- This should conform to GAP.
- Recommended pneumatic with the right nozzle type or motorized sprayers which ensure uniform distribution of the product over the entire plot should be used.
- The farmer should be encouraged to use personal protective equipment (PPE)

4.2 Second Year Large-Scale, Researcher/Farmer-Managed Trials

If the product does not give consistent results in the first year, a second year large scale trial should be conducted.

5.0 Observations and Data Collection, Recordings and Measurements

- Number of target pests from the ground to hand-height (about 2m) on all cocoa trees in each plot should be assessed prior to application of the insecticides and repeated 48 hours thereafter.
- The numbers of the target pest should be recorded every month.

5.1 Meteorological Data

- Collect meterological data as described earlier.

5.2 Edaphic/Soil Data

- Collect Edaphic/soil data as described earlier.

6.0 Residue Trials

- Three 0.4ha plots should be sprayed monthly from August to December for most of the insect pest species excluding November on farmer's farm.
- The dosage used should be 1.5 times the pre-determined effective dosage in 55 litres of water per hectare of mature cocoa.
- Use recommended application equipment such as a mist blower.
- Pods from all plots, including the controls, should be harvested 1, 7, 14, 21 and 28 days after the last product application.
- The beans should be fermented for 6 days, using the heap method or any appropriate method and sun-dried for 10-14 days.
- The dry beans from the three replicates of the treated plots should be bulked together and a sample taken for the residue analysis.
- A similar one bulk sample from the untreated plots should also be taken for the residue analysis at any accredited residue analysis laboratory.

7.0 Results

The results should be presented in systematic and easily understandable form and should be subjected to statistical analysis using standard methods. The analyzed data should be interpreted and appropriate recommendations made.

CHAPTER 7

PROTOCOL FOR BIOLOGICAL EVALUATION OF INSECTICIDE TO CONTROL THE OIL PALM LEAF MINER

Introduction

This Framework Protocol has been prepared to facilitate the conduct of field trials and effective comparison of test results in all Member States of ECOWAS, CILLS and UEMOA.

This section defines the general principles for biological evaluation of formulation of insecticide to control the oil palm leaf miner.

Approvals and Amendments

Initial Approval by WAPRC:… under Framework Protocol No. PC2

1.0 Experimental Conditions

1.1 Target Pest, Selection of Crops and Cultivar/varieties

The oil palm leaf miner *Coelaenomenodera lameensis* Berti and Mariau (Coleoptera: Chrysomelidae) is the key pest of oil palm in the ECOWAS Sub-region.

The other species is *Coelaenomenodera elaeidis.*

The relevant plant is oil palm *Elaeis guineensis* Jac. which is the most widely cultivated palm in the sub-region. The other *Elaeis oleifera* formerly *E. melanoccoca* (exotic) is mainly used for experimental purposes.

1.2 Test Conditions

- The test conditions should be favourable for the development of the pathogen.
- The test should be carried out in areas where there is sufficient presence of the pathogen.
- The agronomic practices should be uniform in all the test plots and consistent with Good Agricultural Practices (GAP) under local conditions.
- The cropping condition (soil type, fertilizer application, tillage, cultivar, row spacing) should be uniform in all the test plots and consistent with GAP under local conditions.
- The cropping history and the pesticide products applied in the preceding two years must be known.
- The test should be conducted in similar or distinct agro-climatic conditions, preferably in different years or growing seasons.
- The exact number of tests to be conducted will be as spelt out in the harmonized document containing pesticides registration requirements in Member States of ECOWAS, CILLS and UEMOA.

1.3 Experimental Procedures and Conduct of Tests

The experimental procedure for test will depend on the cultivar/variety, pest species, method of treatment and the application equipment used.

1.3.1 On-site test

- Treatments should consist of the test product, reference product and an untreated control.
- The plots should be laid out in a Randomized Complete Block Design with 4 replications.
- There should be at least 16 trees per plot and records taken on 4 trees.
- Each plot should be separated by 4 inter-rows as buffer.

1.3.2 Test in local farming condition

The number of trees per plot should be between 10-16 trees per 0.5 - 1 ha, respectively at sites where pest pressure is high.

2.0 Application of Treatment

2.1 Product(s) Under Study

- The product to be tested should be a formulated product and should have a specific name, the Material Safety Data Sheet, technical leaflet and draft label.
- In view of the variability of conditions under which pesticides are used, it is necessary to include a reference product in field trials to allow meaningful evaluation of efficacy under the conditions of the trial.
- Satisfactory levels of efficacy will generally be met when performance is comparable to that of the reference product which should preferably be a registered product widely accepted as satisfactory in practice. However, other considerations (e.g. manner of use, side effects, etc) may arise in assessing what is a satisfactory level of efficacy.
- Where the type of pesticide or its use is new, comparison with a reference product may be impossible or inappropriate. In this case, the product under study should show a consistent well-defined benefit.
- The test product should effectively reduce the pest population and the damage below an economic or phytosanitary threshold level, where this is known.
- Where feasible the reference product chosen should be one which has shown satisfactory results in practice and its mode of action should be the same as or similar to that of the test product. The registration authority must be consulted on issues relating to the selection of a reference product for the study. Where there is the need to use a reference product other than the one agreed or recommended by the registration authority, a justification must be made. Registration authorities must assist test Agents to secure appropriate reference products if it becomes necessary.

2.2 Untreated Control

An untreated control is needed for all tests conducted.

2.3 Application Methods

Application should conform to GAP.

2.3.1 Method of application

The recommended method of application should be used. The method of application should be chosen depending on the size of the plantation, mode of action of pesticide and plant height.

2.3.2 Type of application equipment

- , Knapsack mist blowers of about 35-50 cc can be used for trials on short plants up to 6 m.
- Fogging or tractor mounted mistblower are recommended for large scale plantations with tall plants,
- Trunk injection using a driller and injector can be used on limited scale if the pesticide is systemic.

2.3.3 Time and frequency of application

Treatments must be applied when the pest pressure is high i.e. external adult live index of 2 (*number of external adults per sampled fronds*), and above from sampling results.

2.3.4 Application rate and volumes

- The product should be tested using the recommended rate(R).
- The rate should be expressed in kg/ha or l/ha of the formulated product.
- It may also be necessary to express this in g a.i/kg or g a.i/l.
- A minimum of three rates should be tested on-site; that is, the rate recommended by the manufacturer, a lower rate and a higher rate.
- The exact choice of rate is expected to help determine whether the rate recommended by the manufacturer is the optimal in terms of efficacy and economic returns under any given agro-ecological condition.
- In local farming settings, the optimal rate ascertained from on-site tests should be tested.
- The volume sprayed per plant should be indicated. The dose administered for very high vapour pressure products (fumigants, aerosols and vapourizers), should be expressed in per square metre of surface area, and per cubic metre of volume eg in a greenhouse.

2.3.5 Information on all other pesticide products

Where other pesticide products (or biological control agents) are used

- They should be applied uniformly on all the plots.

- They should not be mixed with the product under study and the reference product.
- The exact time, interval and number of applications should be recorded.

3.0 Observations, Data collection and Measurements

3.1 Meteorological Data

- The data obtained from the test site or from the nearest meteorological station should be recorded. Such data should include rainfall/precipitation (frequency and volume) and temperature (mean, maximum and minimum in ^{O}C).
- All significant changes in the weather should be noted, particularly those likely to affect the test (hygrometry, dry spells).
- Precise data on all possible irrigation of the area should be indicated.

3.2 Edaphic data

Not required.

3.3 Method, Time and Frequency of Observation

The stage of growth of the plant should be recorded at each application time.

3.3.1 Method of observation

The sampling or observation method depends on the pest under consideration. The method chosen should be such that statistical analysis can be conducted to assess the efficacy of the product.

3.3.2 Time and frequency of data collection

- Data should be collected 24hours before and after product application. .
- Subsequently, every week for 12 weeks.
- The number of larvae, pupae, internal and external adults should be counted.

Note of caution: If the baseline data show increase after chemical application, a second application may be necessary within 21 days. If the increase trend continues, then the experiment must be discontinued within 5 weeks and data analyzed and recommendations made.

Effect on yield: Effect on yield may be realized in 2 years provided the pest is effectively controlled.

3.4 Observation of Phytotoxicity

Observe and record all effects of phytotoxicity if trunk injection or root absorption was carried out.

3.5 Observation of Effects on Non-Target Organism

3.5.1 Effects on other pest

All observed effects on other pest, be them positive or negative, should be recorded.

3.5.2 Effects on beneficial organisms

Any positive or negative effect on parasitoids and predators, pollinators on adjacent or succeeding crops should be recorded. Any effect on the environment especially the effects on fauna (both terrestrial and acquatic) should be described.

3.6 Quantitative and Qualitative Evaluation of Harvested Crop

Although it is recognized that crop yield depends on several factors, quantitative and /or qualitative assessment of harvested crop is required. Such evaluation could include taint, residue and physical appearance in accordance with national standards.

4.0 Results

The results should be presented in systematic and easily understandable form and should be subjected to statistical analysis using standard methods. The analyzed data should be interpreted and appropriate recommendations made. (*Vide* OEPP PP 1/152 (2) Standard Directive for the conduct and analysis of biological evaluation tests and OEPP PP 1/181 standard –Directive on biological evaluation of phytosanitary products for the conduct of biological evaluation tests and presentation of reports).

CHAPTER 8

PROTOCOL FOR BIOLOGICAL EVALUATION OF INSECTICIDES AGAINST MAJOR PESTS OF VEGETABLES

Introduction

This Framework Protocol has been prepared to facilitate the conduct of field trials and biological evaluation of insecticides to control major pests of vegetables (local and exotic) grown in all Member States of ECOWAS, CILLS and UEMOA.

This section describes the principles for the conduct of biological evaluation test on new active ingredients or insecticide formulations designed to control major insect pest of vegetables.

Approvals and Amendments

Initial Approval by WAPRC: July 2008, Reference No. PC 2 PS 18_ECOWAS

1.0 Experimental Conditions

1.1 Target Pests, Selection of Crops and Cultivar/varieties

The vegetable crops under cultivation are

- Local vegetables: tomato. hot pepper, egg plant (garden eggs and aubergine) and shallot
- Exotic vegetables: Cucumber, pumpkins and squashes, water melon, equsi melon, spring/salad onions, carrots, lettuce, cabbage, cauliflower, radish, turnip, and ammaranthus

The relevant insects are;

Solanaceae: Tomato, egg plant and pepper: leaf miner (*Liriomyza spp*), whitefly (*Bemisia tabaci*), cotton aphid (*Aphis gossypii*), coreid bugs (*Acanthocoris spp*), fruit piercing moth (*Achaea lienardi* Boisd), green shield bugs (*Nezera viridula*), plant bugs (*Nesidiocoris tunuis*), African mole cricket (*Gryllotalpa africana*), large brown cricket (*Brachytrupes orbonalis*), budworm (*Scrobipalga blaspsigona*), egg plant skeletonizer (*Selepa docilis*), stem borer (*Europhera villora*), cocoa mosquito (*Helopeltis begrothi*) and stem and fruit borer (*Leucinodes orbonalis*) termite (*Odontotermites spp*), epilachna beetle (*Epilachna spp*) variegated grasshopper (*Zonoceros variegates*).

Malvaceae (Okra): flea beetle (*Podogrica uniformis*), cotton leaf roller (*Sylepta derogate*), cotton stainer (*Dysdercus spp*), cotton leafworm (*Spodoptera littoallis*), green shield bugs (*Nezera viridula*), jassids (*Empoasca spp*), variegated grasshopper (*Zonoceros variegatus*), seed bugs (*Oxycarenus spp*), spiny bullworm (*Erias biplaga*) and cotton semi-looper (*Anomis flava*)

Brassica or cole crops: Diamondback moth (*Plutella maculipennis*), cabbage aphid (*Brevicoryne brassicae),* cabbage webworm or bud worm (*Hellula undalis*), cabbage sawfly (*Athalia sjostedti*) and cabbage flea bettle (*Phyllotreta spp*).

Onions and shallots: Onion thrips (*Thrips tabaci*), blister beetle (*Coryna hermannaiae*) and cotton leafworm (*Spodoptera littoralis*)

Cucurbits: Aphids, leaf footed bugs (*Leptoglossus australis*), melon fly (*Dacus curcubitae*), epilachna beetle (*Epilachna spp*)

Lettuce: Cutworms (*Agrotis spp*), green semilooper (*Plusia signata*) and cotton leaf worm *(Spodoptera litorallis)*

1.2 Conditions for Trials

- The test should be carried out in areas where the pest pressure is generally high to ensure that high pest infestation coincides with the different stages of the crops.
- The cropping condition (type of soil, fertilizer application, tillage, cultivar, row spacing) should be uniform in all the test plots and consistent with Good Agriculture Practices (GAP).
- The test should also take into account the different pest species and their stages of development. The cropping history and pesticide products applied in the preceding two years must be known and recorded.
- The test should be conducted in similar or distinct agro-climatic conditions preferably in different years or growing seasons under local farming conditions.
- It is important to conduct at least three independent tests in each agro-ecological zone (Sahel, Sudan and Guinea etc).
- For the purpose of obtaining provisional registration, the test should be conducted for two years on-site and for one year in on-farm as spelt out hereunder:

Year One: On-station test. Trial should be conclusive with reliable results.

Year Two: On-station and on-farm test, taking the different agro ecological zones into consideration.

For the purpose of registration, two additional tests in on-farm conditions are necessary. It is proposed that the tests should be conducted by different institutions or individuals accredited by WAPRC.

1.3 Experimental Procedures and Conduct of Tests

1.3.1 On-station test

Treatments:

- The treatments consist of different rates (generally three) of the product under study (the rate of the manufacturer, $^3/4$ and 3/2 of these rates), a reference product known for its efficacy against vegetable crop pests and with similar mode of action to that of the product under study and an untreated control.
- The experimental design should be Randomised Complete Block Design.
- The plot size should generally be 25 m^2 with 3 m spacing between the plots, but evaluation of damage will be done only on the middle plants. At least four (4) replications are required for each treatment.

1.3.2 On-farm test

Treatment:

- The treatments are generally made up of three rates. The best rate of the product derived from on-site tests, the rate proposed by the manufacturer and an untreated control.

- The plot size should be generally be 100 m^2. Owing to the wide variations between the plots and the possibility of losing some of the treatments, at least 5 replications are required for every test.
- Adequate spacing should be provided (3 m) between the plots taking into account the wind direction during treatments.

2.0 Application of Treatment

2.1 Product(s) understudy

The product to be tested should be a formulated product and should have a specific name.

2.2 Reference Product

In view of the variability of conditions under which pesticides are used, it is necessary to include a reference product in field trials to allow meaningful evaluation of efficacy under the conditions of the trial. Satisfactory levels of the efficacy will generally be met when performance of the test product is comparable to that of the reference product, which should preferably be a registered product widely accepted as effective and satisfactory in practice. However, other considerations (e.g. manner of use, side effects, etc) may arise in assessing what is a satisfactory level of efficacy.

Where the type of pesticide product or its use is new, comparison with a reference product may be impossible or inappropriate. In this case, the product under study should show consistent well-defined benefits. The pesticide to be introduced should be able to bring and/or keep the pest population and the damage to which it gives rise below an economic or phytosanitary threshold level, where this is known.

Wherever feasible the reference product chosen should be one which has shown satisfactory results in practice and its mode of action should be the same as or similar to that of the test product. The registration authority must be consulted on issues relating to the selection of a reference product for the study. Where there is the need to use a reference product other than the one agreed or recommended by the registration authority, a justification must be made.

2.3 Untreated Control

An untreated control is needed for all trials both on-site tests and on-station or on-farm.

2.4 Application Methods

Application should conform to good agricultural practice (GAP).

2.4.1 Method of application

The recommended method of application should be used.

2.4.2 Type of application equipment

Each application should be done with equipment that ensures uniform distribution of the product over the entire plot or that ensures guided target treatment. Factors likely to modify efficiency of delivery such as pressure or nozzle type should be avoided. The equipment should be selected in accordance with product recommendation. Proper calibration of the application equipment is essential for the correct delivery of the chemical.

2.4.3 Timeframe and frequency of application

The number of applications and date of each treatment should be consistent with those indicated for the proposed usage. These will depend on the stage of development of the

crop and the biology of the target pest (s) such as stage of development, tolerance threshold and peak of population. The date of the applications should be recorded.

2.4.4 Application rate and volumes

On-site: a minimum of three rates should be tested; the manufacturer's rate, a lower and a higher rate. The exact choice of rate is expected to help determine whether the rate recommended by the manufacturer is the optimal rate in terms of efficacy and economic returns under a given agro-ecological conditions.

In on-farm setting, the optimal rate determined from on-site test is generally tested.

The applicable rate should be expressed in g ai/ha and in kg/ha or 1/ha of the formulated product. For liquid formulations, the concentration should be in g ai/l. It may also be necessary to express this dose in g ai/kg or g.ai/1. For formulations in powder for dust spraying, be it granulated or similar products; this should be expressed in g ai/kg or %.

For seed treatment, the rate should be expressed in kg (or litres) of the formulated product per ton of seeds, and in g ai/kg of seeds.

The actual rate applied should always be measured and any deviation from the recommended rate recorded.

2.4.5 Information on all other pesticide products

Where other pesticide products (or biological control agents) are used, they should be applied uniformly on all plots. They should not be mixed with the product under study and the reference product. The date on which the treatments were applied should be recorded. The risk of interference should be minimized.

3.0 Observations and Data Collection, Recordings And Measurements

3.1 Meteorological Data

Days before and after product application, the meteorological data likely to influence crop development and/or that of the target pest and the action of the pesticide product should be recorded. Such data should normally include rainfall and temperature. All data should in principle, be recorded on the test site, especially for on-site test. It is sometimes difficult to obtain meteorological data in the local on-farm condition in which case, the relevant data could be obtained from the nearest meteorological station.

On the day of application, the meteorological data likely to influence the quality and persistency of the product should be recorded. Such data will normally include at least rainfall (nature and volume in mm) and temperature (mean maximum and minimum in °C). Any significant change in the day's weather should be recorded and its relation to the application should also be indicated. Throughout the test period, unusual weather conditions such as prolong dry periods, heavy rains, strong winds etc, likely to influence the outcome of the test should be recorded. Accurate data should be provided on any irrigation to be carried out on the plot.

3.2 Edaphic Data

These are not required.

3.3 Method, Time and Frequency of Observation

The age and growth stage of the crop should be recorded during each application.

3.3.1 Method of observation

The sampling method or observation method should depend on the pest under consideration. The method chosen should lend itself to statistical evaluation to assess the efficacy of the product. The sampling period should be in the mornings between 6.00-8 00 am and evenings between 5.00-6.00 pm. The population of the pests that cause damage to the different growth stages of the plants should be recorded in order to assess the effect of the product on the abundance of the target pest (s). The level of pest infestation should be quantified by determining the percent leaf, fruit or head damage in the various treatments. Leaf/fruit damage can also be classified according to the rating scale of 0-7. Where 0 = 5%; 1 = 15%: 2 = 25%; 3 = 35%; 4 = 45%; 5 = 60%; 6 = 85% and 7 = 100%. Other parametres such as dates of 50% emergence and 50% flowering should be recorded.

3.3.2 Timeframe and frequency of observation

At least one observation is required on pest density and damage (generally 1-3 days) before the first treatment during the growing season. A sampling should be carried out to determine species composition and degree of infestation. About 2-3 days after treatment, sampling should be taken to assess the level of population in relation to the initial pre-treatment. The timeframes and frequencies of observation should take into account the phonological stage of the crop and the level of pest population, stage of development of the pest and the rate of product action and persistence.

3.4 Observations of Phytotoxicity

Plants must be examined for the presence or absence of phytotoxicity on/from the leaves. Where toxicity effects are measurable, count the number of plants/leaves showing the symptoms. The results must be indicated in absolute figures. Otherwise, the damages caused by phytotoxicity can be estimated based on a rating scale as against the untreated plots. Symptoms of burning such as chlorosis, faltering, deformation etc. must be well described.

3.5 Observations of Effects on Non-target Organisms

3.5.1 Effects on other pests

All observed effects on other pests whether positive or negative should be recorded.

3.5.2 Effects on beneficial organisms

Any positive or negative effects observed on parasitoids and predators, pollinators on adjacent or succeeding crops should be recorded. Any effect on the environment especially the effects on other fauna (both terrestrial and aquatic) should be recorded.

3.6 Quantitative and Qualitative Evaluation of Harvest

Although it is recognized that crop yield depends on several factors, quantitative and/or qualitative assessment of harvested produce is required. Record the width, length, and weight of the harvested crop. Weight of marketable fruits/heads and the final yield in tonnes or kilograms per ha should be estimated.

4.0 Results

Test results should be presented in systematic and easily understandable form. They should be subjected to statistical analysis using pre-established and reliable methods. The report should include analysis and interpretation of the relevant data. Other stages of the evaluation will then follow. *Vide* OEPP PP1/152 (2) Standard Directive for the conduct and analysis of biological evaluation tests and OEPP PP 1/181 Standard-

Directive on biological evaluation of phytosanitary products for the conduct of biological evaluation tests and presentation of reports.

CHAPTER 9

PROTOCOL FOR THE ASSESSMENT OF BIOLOGICAL EFFICACY OF FUNGICIDES AGAINST ROOT ROT BLIGHT COMPLEX (APOLLO DISEASE) OF COCOYAM

Introduction

This specific protocol has been prepared to facilitate the conduct of field trials and effective comparison of test results in all Member States of ECOWAS, CILSS and UEMOA.

This section defines the general principles for biological evaluation of new active ingredients or formulations of fungicides to control fungal diseases on cocoyam.

Approvals and Amendments

Initial Approval by WAPRC: ..... Reference No. ECOWAS.

1.0 Experimental Conditions

1.1 Target Pest, Selection of Crops and Varieties

The major root rot blight complex organisms are *Pythium myriotylum* (Dreschsler), *Pythium spp*., *Fusarium solani* (Mart) Sacc, *Rhizoctonia solani* Kuhn.

- The fungal sps attack the roots at any stage if they are planted in poorly drained soils. The entire root system is destroyed except for a few apparently healthy roots nearest to the soil surface. The interior of the corm becomes a soft mass of dissociated cells. The corky layer of the corm remains intact until the interior of the corm has completely deteriorated. Warm moist soil conditions favour disease development.
- The crop under cultivation is Cocoyam (*Collocassia spp. and Xanthosoma spp.)*
- It is important to take into consideration the biology and ecology of each species used in the conduct of the experiment. Thus, the test should be conducted when there is high disease pressure of the organism concerned, to ensure that the high infestation period coincide with the stages.

1.2 Test Conditions

- The test conditions should be favourable for the development of the pathogen. The agronomic practices should be uniform in all the test plots and consistent with Good Agricultural Practices (GAP) under local conditions.
- The test should be carried out in areas where the disease pressure is generally high. The cropping condition (soil type, fertilizer application, tillage, cultivar, row spacing) should be uniform in all the test plots.. The cropping history and the pesticide products applied in the preceding two years must be known.
- The test should be conducted in similar or distinct agro-climatic conditions, preferably in different years or growing seasons. The exact number of tests to be conducted will be spelt out in the harmonized document containing pesticides registration requirements in Member States of ECOWAS, CILLS and UEMOA.

1.3 Experimental Procedures and Conduct of Tests

- The test should comprise at least three (3) treatments for the test product the reference product and the control.
- The experimental design should be a Randomised Complete Block Design (RCBD) with four replications. Enough space should be provided between the blocks and between the plots. The minimum size of the plots should be $50m^2$

2.0 Application of Treatment

2.1 Product(s) Under Study

- The product to be tested should be a formulated product and should have a specific name.

2.2 Reference Product

- In view of the variability of conditions under which pesticides are used, it is necessary to include a reference product in field trials to allow meaningful evaluation of efficacy under the conditions of the trial. Satisfactory levels of efficacy will generally be met when performance is comparable to that of the reference product, which should preferably be a **registered product** widely accepted as satisfactory in practice. However, other considerations (e.g. mode of application, side effects, etc) may arise in assessing what is a satisfactory level of efficacy.
- Where the type of pesticide product or its use is new, comparison with a reference product may be impossible or inappropriate. In this case, the product under study should show a consistent well-defined benefit. The pesticide to be introduced should be able to bring and/or keep the disease intensity and the damage to which it gives rise below an economic or phyto-sanitary threshold level, where this is known.
- Wherever feasible the reference product chosen should be one which has shown satisfactory results in practice and its mode of action should be the same as or similar to that of the test product. The registration authority must be consulted on issues relating to the selection of a reference product for the study. Where there is the need to use a reference product other than the one agreed or recommended by the registration authority, a justification must be made.

2.3 Untreated Control

An untreated control is needed for on-station tests as well as test conducted under local farming conditions.

2.4 Application Methods

Applications should conform to GAP

2.4.1 Method of Application

The recommended method of application should be used and specified. (e.g.: spraying, fumigation, drenching and/or incorporation of granules into the soil).

2.4.2 Type of Application Equipment

The type of application equipment used should be specified. In all cases, the application should be done with the aid of equipment that ensures uniform distribution of the

product over the entire plot or that which ensures guided on-target treatment. Factors likely to affect the efficiency of delivery (pressure and type of nozzle) should be avoided.

2.4.3 Timeframe and Frequency of Application

The number of applications and the date of each application should be as recommended for the proposed usage. These will depend on the objective of the evaluation and should be consistent with the stage of development of the crop and the target organism.

2.4.4 Application Rates and Volumes

- The product should be tested using the recommended rate (R). The rate should be expressed in kg/ha or l/ha of the formulated product. It may also be necessary to express this in g.ai/kg or g.ai/l.
- A minimum of three rates should be tested on site; that is, the rate recommended by the manufacturer, a lower rate and a higher rate. The exact choice of rates is expected to help determine whether the rate recommended by the manufacturer is the optimal in terms of efficacy and economic returns under any given agro-ecologic condition.
- In local farming setting, the optimal rate ascertained from on-station tests is generally tested.
- For liquid formulation, the volume per plant should also be indicated.

2.4.5 Information on all other pesticide products

Where other pesticide products (or biological control agents) are used, they should be applied uniformly on all the plots. They should not be mixed with the product under study and the reference product. The exact dates on which such treatments were applied should be recorded.

3.0 Observations, Data Collection and Measurements

3.1 Meteorological and Edaphic Data

3.1.1 Meteorological Data

The data obtained from the test site or from the nearest meteorological station should be specified. Such data should include precipitations (frequency and volume) and temperature (mean, maximum and minimum in ^{o} C). All significant changes in the weather should be noted, particularly those likely to affect the test (humidity, dry spells). Precise data on all possible irrigation of the area should be indicated.

3.1.2 Edaphic Data

During conduct of the test, it will be useful to be aware of the soil pH (10-20 cm) structure and texture (granulometry and organic content).

3.2 Method, Time and Frequency of Observations

3.2.1 Method

Data on seedling emergence, vigor, density and the incidence and severity of infection should be recorded. The rating scale of 1-5, should be used and specified. The growth stage of crop should be noted during each application.

Rating scale:

1: No visible symptoms

5: Very severe damage beyond recovery

3.2.2 Timeframe and Frequency of Observation

The timeframe and frequency of the observation should be specified: the first evaluation should be conducted just before the treatment; followed by one or several intermediary evaluations and a final evaluation.

3.3 Observations of Phytotoxicity on Crop

All positive effects of the treatment on the plants should be noted. The crop should be examined for the presence or absence of phytotoxic effects. In case of phytotoxicity, the symptoms (stunting, chlorosis, deformation, etc) should be described.

3.4 Observations of the Effects on Non-Target Organisms

3.4.1 Effects on other disease organisms

All observed effects on other diseases either positive or negative should be noted.

3.4.2 Effects on beneficial organisms

Any positive or negative effects observed on parasitoids and predators, pollinators present naturally or introduced, on adjacent as well as succeeding crops, should be noted. Any effect on the environment, especially the effects on flora and fauna, should be described.

3.5 Quantitative and Qualitative Evaluation of Harvested Crop

The yield and quality of the harvested produce should be assessed. The yield assessment methods and the quality index measurement standards used should be described.

4. 0 Results

Test results should be presented in systematic and easily understandable form. They should be subjected to appropriate statistical analysis. The report will include analysis and interpretation of the relevant data. Other stages of the evaluation will then follow. *Vide* OEPP PP 1/152 (2) standard Directive for the conduct and analysis of biological evaluation tests and OEPP PP 1/181 standard - Directive on biological evaluation of phytosanitary products for the conduct of biological evaluation tests and presentation of reports.

CHAPTER 10

PROTOCOL FOR BIOLOGICAL EVALUATION OF INSECTICIDES AGAINST MAJOR PESTS OF ROOT AND TUBER CROPS

Introduction

This Framework Protocol has been prepared to facilitate the conduct of field trials and biological evaluation of insecticides to control major pests of root and tuber crops grown in all Member States of ECOWAS, CILLS and UEMOA.

This section describes the principles for the conduct of biological evaluation test on new active ingredients or insecticide formulations designed to control major pest of root and tuber crops.

Approvals and Amendments

Initial Approval by WAPRC: …., Reference No. ……_ECOWAS

1.0 Experimental Conditions

1.1 Target Pests, Selection of Crops and Cultivar/varieties

The roots and tuber crops concerned are:

- Cassava (*Manihot esculenta*)
- Yam (*Dioscora spp)*
- Sweetpotatoe (*Ipomea batata)*

The relevant insect pests are;

- **Cassava**: cassava mealybugs *(Phenacoccus manihoti*), whitefly (*Bermisia tabaci*), variegated grasshopper (*Zonoceros variegatus*),
- **Yam:** yam mealybugs (*Dysmicoccus brevipes*), yam tuber beetles (*Heteroligus spp*).aphids (refer name)
- **Sweetpotato**: weetpotato butterfly (*Acraea acerata*), sweetpotato weevils (*Cylas puncticollis*, *Cylas fermicarius)*, white fly, (*Bermisia tabaci*), *Alcidodes sp(vine beetle)*

1.2 Conditions for Trials

- The test should be carried out in areas where the pest pressure is generally high to ensure that high pest infestation coincides with the different stages of the crops.
- The cropping condition (type of soil, fertilizer application, tillage, cultivar, row spacing) should be uniform in all the test plots and consistent with Good Agriculture Practices (GAP).
- The test should also take into account the different pest species and their stages of development. The cropping history and pesticide products applied in the preceding two years must be known and recorded.
- The test should be conducted in similar or distinct agro-climatic conditions preferably in different years or growing seasons under local farming conditions.

- It is important to conduct at least three independent tests in the appropriate agro-ecological zone within the humid sub-region.
- For the purpose of obtaining provisional clearance , the test should be conducted for two years on-station/site and for one year in on-farm as spelt out hereunder:

Year One: On-station/site test. Trial should be conclusive with reliable results.

Year Two: On-station and on-farm test, taking the different agro-ecological zones into consideration.

It is proposed that the tests should be conducted by different institutions or individuals accredited by WAPRC.

1.3 Experimental Procedures and Conduct of Tests

1.3.1 On-station test

Treatments:

- The treatments consist of different rates (generally three) of the product under study (the rate of the manufacturer, $^3/4$ and 3/2 of these rates), a reference product known for its efficacy against root and tuber crop pests and with similar mode of action to that of the product under study and an untreated control.
- The experimental design should be Randomised Complete Block Design.

The plot size should generally be $50m^2$ with 3m spacing between the plots, but evaluation of damage will be done only on the middle plants. At least four (4) replications are required for each treatment.

1.3.2 On-farm test

Treatment:

- The treatments are generally made up of three rates. The best rate of the product derived from on-site tests, the rate proposed by the manufacturer and an untreated control.
- The plot size should generally be 100 m^2. Owing to the wide variations between the plots and the possibility of losing some of the treatments, at least 5 replications are required for every test.
- Adequate spacing should be provided (4 m) between the plots taking into account the wind direction during treatments.

2.0 Application of Treatment

2.1 Product(s) Under Study

The product to be tested should be a formulated product and should have a specific name.

2.2 Reference Product

In view of the variability of conditions under which pesticides are used, it is necessary to include a reference product in field trials to allow meaningful evaluation of efficacy under the conditions of the trial. Satisfactory levels of the efficacy will generally be met when performance of the test product is comparable to that of the reference product, which should preferably be a registered product widely accepted as effective and satisfactory in

practice. However, other considerations (e.g. manner of use, side effects, etc) may arise in assessing what is a satisfactory level of efficacy.

Where the type of pesticide product or its use is new, comparison with a reference product may be impossible or inappropriate. In this case, the product under study should show consistent well-defined benefits. The pesticide to be introduced should be able to bring and/or keep the pest population and the damage to which it gives rise below an economic or phytosanitary threshold level, where this is known.

Wherever feasible the reference product chosen should be one which has shown satisfactory results in practice and its mode of action should be the same as or similar to that of the test product. The registration authority must be consulted on issues relating to the selection of a reference product for the study. Where there is the need to use a reference product other than the one agreed or recommended by the registration authority, a justification must be made.

2.3 Untreated Control

An untreated control is needed for all trials both on-site test and on-station or on-farm.

2.4 Application Methods

Application should conform to GAP.

2.4.1 Method of application

The recommended method of application should be used.

2.4.2 Type of application equipment

Each application should be done with equipment that ensures uniform distribution of the product over the entire plot or that ensures guided target treatment. Factors likely to modify efficiency of delivery such as pressure or nozzle type should be avoided. The equipment should be selected in accordance with product recommendation. Proper calibration of the application equipment is essential for the correct delivery of the chemical.

2.4.3 Timeframe and frequency of application

The number of applications and date of each treatment should be consistent with those indicated for the proposed usage. These will depend on the stage of development of the crop and the biology of the target pest (s) such as stage of development, tolerance threshold and peak of population. The date of the applications should be recorded.

2.4.4 Application rate and volumes

On-station/site: a minimum of three rates should be tested; the manufacturer's rate, a lower and a higher rate. The exact choice of rate is expected to help determine whether the rate recommended by the manufacturer is the optimal rate in terms of efficacy and economic returns under a given agro-ecological conditions.

In On-farm setting, the optimal rate determined from On-station/site test is generally tested.

The applicable rate should be expressed in g.ai/ha and in kg/ha or 1/ha of the formulated product. For liquid formulations, the concentration should be in g.ai/l. It may also be necessary to express this rate in g.ai/kg or g.ai/1. For powdered, granulated, or similar products; this should be expressed in g. ai/kg or %.

For seed treatment, the rate should be expressed in kg (or litres) of the formulated product per ton of seeds, and in g. ai/kg of seeds.

The actual rate applied should always be measured and any deviation from the recommended rate recorded.

2.4.5 Information on all other pesticide products

Where other pesticide products (or biological control agents) are used, they should be applied uniformly on all plots. They should not be mixed with the product under study and the reference product. The date on which the treatments were applied should be recorded. The risk of interference should be minimized.

3.0 Observations and Data Collection, Recordings and Measurements

3.1 Meteorological Data

Days before and after product application, the meteorological data likely to influence crop development and/or that of the target pest and the action of the pesticide product should be recorded. Such data should normally include rainfall and temperature. All data should in principle, be recorded on the test site, especially for on-site test. It is sometimes difficult to obtain meteorological data in the local on-farm condition in which case, the relevant data could be obtained from the nearest meteorological station.

On the day of application, the meteorological data likely to influence the quality and persistency of the product should be recorded. Such data will normally include at least rainfall (nature and volume in mm) and temperature (mean maximum and minimum in °C). Any significant change in the day's weather should be recorded and its relation to the application should also be indicated. Throughout the test period, unusual weather conditions such as prolong dry periods, heavy rains, strong winds etc, likely to influence the outcome of the test should be recorded. Accurate data should be provided on any irrigation to be carried out on the plot.

3.2 Edaphic Data

The edaphic data to be gathered include soil type (international standards to be specified), pH, organic matter content, soil moisture, soil structure and texture and soil fertility status.

3.3 Method, Time and Frequency of Observation

The age and growth stage of the crop should be recorded during each application.

3.3.1 Method of observation

The sampling method or observation method should depend on the pest under consideration. The method chosen should lend itself to statistical evaluation to assess the efficacy of the product. Sampling should be made three times a week on 4 middle rows of the plots. The samples should comprise 200 leaves (50 leaves per row x 4) from tagged plants at early vegetative stage. The date of 50% emergence and 50% maturity should be recorded. From emergence to maturity, the number of larvae/insects on tagged plants should be counted three times a week. The sampling period should be between 6.00am and 7.00am and between 5.00pm and 6.00pm.
The level of pest infestation should be quantified by determining the percent leaf damage in the various treatments. Leaf damage can also be classified according to the rating scale of 0-7. Where 0 = 5%; 1 = 15%: 2 = 25%; 3 = 35%; 4 = 45%; 5 = 60%; 6 = 85% and 7 = 100%.

3.3.2 Timeframe and frequency of observation

At least one observation is required on pest density and damage (generally 1-3 days) before the first treatment during the growing season. Sampling should be carried out to determine species composition and degree of infestation. About 2-3 days after treatment, sampling should be taken to assess the level of population in relation to the initial pre-treatment. A second observation should be made 5 days after treatment and a third 7 days after treatment. The timeframes and frequencies of observation should take into account the phenological stage of the crop and the level of pest population, stage of development of the pest and the rate of product action and persistence. The observation could be made at weekly intervals.

3.4 Observations of Phytotoxicity

Plants must be examined for the presence or absence of phytotoxicity on/from the leaves. Where toxicity effects are measurable, count the number of plants/leaves showing the symptoms. The results must be indicated in absolute figures. Otherwise, the damages caused by phytotoxicity can be estimated based on a rating scale as against the untreated plots. Symptoms of burning such as chlorosis, faltering, deformation etc. must be well described.

3.5 Observations of Effects on Non-Target Organisms

3.5.1 Effects on other pests

All observed effects on other pests whether positive or negative should be recorded.

3.5.2 Effects on beneficial organisms

Any positive or negative effects observed on parasitoids and predators, pollinators on adjacent or succeeding crops should be recorded. Any effect on the environment, especially, the effects on other fauna (both terrestrial and aquatic) should be recorded.

4.0 Quantitative and Qualitative Evaluation of Harvest

Although it is recognized that crop yield depends on several factors, quantitative and/or qualitative assessment of harvested produce is required. Record the width, length, and weight of the harvested crop. Weight of marketable tubers and the final yield in tonnes or kilograms per ha should be estimated.

5.0 Results

Test results should be presented in systematic and easily understandable form. They should be subjected to appropriate statistical analysis.. The report should include analysis and interpretation of the relevant data. Other stages of the evaluation will then follow. *Vide* OEPP PP1/152 (2) Standard Directive for the conduct and analysis of biological evaluation tests and OEPP PP 1/181 Standard-Directive on biological evaluation of phytosanitary products for the conduct of biological evaluation tests and presentation of reports.

CHAPTER 11

PROTOCOL FOR THE ASSESSMENT OF BIOLOGICAL EFFICACY OF INSECTICIDES AGAINST MAJOR STORAGE PEST OF YAM.

Introduction

This Framework Protocol has been prepared to facilitate the conduct of field trials and biological evaluation of insecticides to control major storage pests of Yam grown in all Member States of ECOWAS, CILLS and UEMOA.

This section describes the principles for the conduct of biological evaluation test on new active ingredients or insecticide formulations designed to control major storage pest of Yam.

Approvals and Amendments

Initial Approval by WAPRC: ……Reference No …….ECOWAS

1.0 Experimental Conditions

1.1 Target Pests, Selection of Crops and Cultivar/varieties

The crop concerned is yam (Dioscorea spp)

The major storage pests concerned are yam beetles, coffee bean weevil,(*Araecerus fasciculatus)* (DeGeer), cornsapp beetle (*Carpophilus dimidiatus* (Fabricius), freeman's sap beetle (*Carpophilus freemani)* and depressed flour beetle(*Palorus subdepressus),*

These beetles infest yam in storage. They make small feeding holes and the adults as well as the larvae tunnel through the tubers. The tissue of the infested tubers become dark brown and the product becomes commercially unattractive. Yam tubers damaged during harvest are more easily attacked. Severe beetle infestations may result in yam tubers completely reduced to black powder especially when stored for a long time. Yam should be stored either on platforms in farmer's field or traders warehouses.

1.2 Conditions for Trials

- The trial should be conducted in localities where yams are cultivated or sold and storage must conform to local practices.
- Tubers must be neatly arranged preferable on wooden platforms, or using local storage practices.
- The tubers selected must be those with high susceptibility to pest attack. The origin of tubers, as well as the applications of agro-chemical products done during the vegetative stage of the crop must be recorded.
- The trial must be conducted in the laboratory or in a storage area of the farmer or trader.
- The test should be carried out in areas where the pest pressure is generally high to ensure that high pest infestation coincides with the different stages of the crops.
- The storage condition (sanitation, ventilation, light, and spacing) should be uniform and consistent with GAP.

- The test should also take into account the different pest species and their stages of development. The cropping history and pesticide products applied in the preceding two years must be known and recorded.
- The test should be conducted in similar or distinct agro-climatic conditions preferably in different years or growing seasons under local farming conditions.
- It is important to conduct at least three independent tests in each agro-ecological zone

Year One: On-station/site trial. Trial should be conclusive with reliable results.

Year Two: On-station/site and On-farm trial , taking the different agro ecological zones into consideration.

For the purpose of registration, two additional trials under On-farm conditions are necessary. It is proposed that the trials should be conducted by different institutions or individuals accredited by WAPRC.

1.3 Experimental Procedures and Conduct of Trials

1.3.1 On-station/site trial

Treatment: The treatments are made up of the test product, a reference product, and an untreated control and a traditional product (if applicable). The experimental design should be complete randomized blocks (CRD). There should be a minimum of 20 tubers of similar size grouped in the same place. There should be a minimum of 2m space between the groups to minimize cross contamination.
There should be six treatments each with four replications.

- T1: untreated control
- T2: Treatment with a traditional product (if applicable),
- T3: Reference product
- T4: Test Product at recommended rate
- T5. Higher rate
- T6. Lower rate

1.3.2 On-farm/warehouse trial

Treatment: The treatments are made up of the test product, a reference product, and an untreated control and a traditional product (if applicable). The experimental design should be complete randomized blocks (CRD). There should be a minimum of 50 tubers of similar size grouped in the same place. There should be a minimum of 2m space between the groups to minimize cross contamination

There should be six treatments each with 6 replications.

- T1: untreated control
- T2: Treatment with a traditional product (if applicable),
- T3: Reference product
- T4: Test Product at recommended rate
- T5. Higher rate
- T6. Lower rate

2.0 Application of Treatment

2.1 Product(s) Under Study

- The product to be tested should be a formulated product and should have a specific name.

2.2 Reference Product

In view of the variability of conditions under which pesticides are used, it is necessary to include a reference product in field trials to allow meaningful evaluation of efficacy under the conditions of the trial. Satisfactory levels of efficacy will generally be met when performance is comparable to that of the reference product, which should preferably be a registered product widely accepted as satisfactory in practice. However, other considerations (e.g. manner of use, side effects, etc may arise in assessing what is a satisfactory level of efficacy.

Where the type of pesticide product or its use is new, comparison with a reference product may be impossible or inappropriate. In this case, the product under study should show a consistent well-defined benefit. The pesticide to be introduced should be able to bring and/or keep the pest population and the damage to which it gives rise below an economic or phytosanitary threshold level, where this is known.

Wherever feasible the reference product chosen should be one which has shown satisfactory results in practice and its mode of action should be the same as or similar to that of the test product. The registration authority must be consulted on issues relating to the selection of a reference product for the study. Where a there is the need to use a reference product other than the one agreed or recommended by the registration authority, a justification must be made.

2.3 Untreated Control

An untreated control is needed for On-station/site trials. Trials should be conducted under local farming conditions. The traditional storage practice used by farmers in the traditional setting could be chosen as the control where applicable.

2.3.1 Traditional method of treatment

Many farmers use natural products to protect tubers against the attacks of insects in storage. Such products must be taken into account during trials at both On –station/site and On-farm or traders storage houses.

2.4 Application Methods

Applications should conform to GAP.. Products must be applied as uniformly as possible on tubers.

2.4.1 Method of *Application*

The method of application should be that which is recommended and should be specified (e.g. spraying, dipping, fumigation and/or dust application).

2.4.2 Type of *Application Equipment*

The type of application equipment used should be specified. In all cases, the application should be done with the aid of equipment that ensures uniform distribution of the product or that which ensures guided on-target treatment. Factors likely to affect the efficiency of delivery (pressure and type of nozzle) should be selected in accordance with product recommendations.

Tubers should be treated with equipment that allows for uniform distribution of the product in accordance with recommended practice.

2.4.3 Timeframe and Frequency of *Application*

The number of applications and the date of each application should be as recommended for the proposed usage. This will depend on the objective of the evaluation and should be consistent with the stage of development of the tubers and the target organism. However, a minimum of 2 to 3 applications of 30 days interval is required during the storage of the tubers. The first application will be done on first day of storage or from the beginning of pest attack.

2.4.4 Application Rates and Volumes

The product should be tested using the recommended rate(R). The rate should be expressed in kg or l of the formulated product per kg of yam tubers. It may also be necessary to express this in g.ai/kg or g. ai/l of tubers. A minimum of three rates should be tested On –station/site; that is, the rate recommended by the manufacturer, a lower rate and a higher rate. The exact choice of rates is expected to help determine whether the rate recommended by the manufacturer is the optimal in terms of efficacy and economic returns under any given agro-ecologic condition.

In local farming setting, the optimal rate ascertained from On-station/site tests is generally tested.

For liquid formulations their concentration (g.ai/l) should also be specified. For very high vapour pressure products (fumigants, aerosols and vaporizers), the rate administered should be expressed in square meter of surface, and per cubic meter volume of warehouse.

2.4.5 Information on all other pesticide products

If other pesticide products (or bio-control agents) are used, they must be uniformly applied within the local (store house, shop, barn etc) and separate from products to be assessed and the reference products. The application dates of these products must be indicated. There must be no cross contamination.

3.0 Observations, Data Collection and Measurements

3.1 Meteorological and Edaphic Data

3.1.1 *Meteorological Data*

The meteorological data likely to affect the action of test product or the population dynamics of the pest in the storage must be recorded during the period of the trial, especially for on-station trials. Such data should include temperature (mean, maximum and minimum in ^{o} C and the relative humidity: minimum average and maximum (in %) at the interior of the storage house or shop.

3.1.2 Edaphic Data

Not required.

3.2 Method, Time and Frequency of Observations

3.2.1 Method

A random sample of 4-5 tubers per treatment per replication should be taken and observed for the presence or absence of damage or holes made by the insect (larvae). Percent (%) tuber infestation in each treatment should be recorded at every observation.

This percentage is determined by counting the number of tubers with holes or damage in relation to the total number of the sampled tubers.

3.2.2 Timeframe and Frequency of observation

The timeframe and frequency of the observation should be specified: the first assessment should be conducted 2-3 days just before treatment, followed by 15 days after the treatment so as to better appreciate the holes/damage and thereafter after every 15 days interval

3.3 Observations of the Effects on Non-Target Organisms

3.3.1 Effects on other pest organisms

All observed effects on other pests including those not targeted, either positive or negative, should be noted.

3.3.2 Effects on beneficial organisms

Any positive or negative effects observed on parasitoids and predators present naturally or introduced, should be noted.

4.0 Results

Test results should be presented in systematic and easily understandable form. They should be subjected to appropriate statistical analysis. The report will include analysis and interpretation of the relevant data. Other stages of the evaluation will then follow. *Vide* OEPP PP 1/152 (2) standard Directive for the conduct and analysis of biological evaluation tests and OEPP PP 1/181 standard - Directive on biological evaluation of phytosanitary products for the conduct of biological evaluation tests and presentation of reports.

CHAPTER 12

PROTOCOL FOR THE ASSESSMENT OF BIOLOGICAL EFFICACY OF FUNGICIDES AGAINST STORAGE ROT OF YAM

Introduction

This specific protocol has been prepared to facilitate the conduct of field trials and comparison of test results in all Member States of ECOWAS, CILSS and UEMOA.

This section defines the general principles for biological evaluation of new active ingredients or formulations of fungicides to control storage rot of yam.

Approvals and Amendments

Initial Approval by WAPRC: ..... Reference No…….ECOWAS.

1.0 Experimental Conditions

1.1 Target Pest, Selection of Crops and varieties

- The major storage rot organisms are *Botryodiplodia theobromae* Pat*, Fusarium spp. Rhizopus nodesus, Rosellinia spp. Aspergillus spp. Penicillium spp.*
- The fungi enter through wounds and natural openings on the surface of the tubers. Optimum rot by the pathogens occurs at 90% relative humidity and at 26–30 °C. The extent of rotting varies with the fungus and the yam species.
- The crop under consideration is yam (Dioscorea *spp.).* The susceptible sensitive cultivar or variety commonly used in the zone should be used for the trial.
- It is important to take into consideration the biology and ecology of each specie used in the conduct of the experiment. Thus the trial should be conducted when there is high disease pressure of the organism concerned.

1.2 Trial Conditions

- The trial conditions should be favourable for the development of the pathogen. The storage practices should be uniform and consistent with GAP under local conditions.
- The trial should be conducted in similar or distinct agro-climatic conditions, preferably in different years. The exact number of trials to be conducted will be spelt out in the harmonized document containing pesticides registration requirements in Member States of ECOWAS, CILLS and UEMOA.

1.3 Experimental Procedures and Conduct of Trial

The trial should comprise at least three (3) treatments for the test product, the reference product and the untreated control.

The experimental design should be a Complete Randomized Design (CRD) with four replications. Enough space should be provided between sets of tubers and the minimum number of matured tubers should be 50.

2.0 Application of Treatment

2.1 Product(s) Under Study

The product to be tested should be a formulated product and should have a specific name.

2.2 Reference Product

In view of the variability of conditions under which pesticides are used, it is necessary to include a reference product in field trials to allow meaningful evaluation of efficacy under the conditions of the trial. Satisfactory levels of efficacy will generally be met when performance is comparable to that of the reference product, which should preferably be a registered product widely accepted as satisfactory in practice. However, other considerations (e.g. manner of use, side effects, etc may arise in assessing what is a satisfactory level of efficacy.

Where the type of pesticide product or its use is new, comparison with a reference product may be impossible or inappropriate. In this case, the product under study should show a consistent well-defined benefit. The pesticide to be introduced should be able to bring and/or keep the pest population and the damage to which it gives rise below an economic or phyto-sanitary threshold level, where this is known.

Wherever feasible the reference product chosen should be one which has shown satisfactory results in practice and its mode of action should be the same as or similar to that of the test product. The registration authority must be consulted on issues relating to the selection of a reference product for the study. Where a there is the need to use a reference product other than the one agreed or recommended by the registration authority, a justification must be made.

2.3 Untreated Control

An untreated control is needed for On-station/site trials as well as test conducted under local storage conditions.

2.4 Application Methods

Applications should conform to GAP.

2.4.1 Method of Application

The recommended method of application should be used and specified (e.g. spraying, fumigation, dust application, etc).

2.4.2 Type of Application Equipment

The type of application equipment used should be specified. In all cases, the application should be done with the aid of equipment that ensures uniform distribution of the product. Factors likely to affect the efficiency of delivery (pressure and type of nozzle) should be avoided.

2.4.3 Timeframe and Frequency of Application

The number of applications and the date of each application should be as recommended for the proposed usage. These will depend on the objective of the evaluation and should be consistent with the stage of development of the crop and the target organism.

2.4.4 Application Rates and Volumes

- The product should be tested using the recommended rate (R). The rate should be expressed in kg/ha or l/ha of the formulated product. It may also be necessary to express this in g.ai/kg or g.ai/l.
- A minimum of three rates should be tested; that is, the rate recommended by the manufacturer, a lower rate and a higher rate. These rates are expected to help determine whether the rate recommended by the manufacturer is the optimal in terms of efficacy and economic returns in any given agro-ecological condition.

2.4.5 Information on all other pesticide products

Where other pesticide products (or biological control agents) are used, they should be applied uniformly. They should not be mixed with the product under study and the reference product. The exact dates on which such treatments are applied should be recorded.

3.0 Observations, Data Collection and Measurements

3.1 Meteorological and Edaphic Data

3.1.1 Meteorological Data

The data obtained from the trial location or from the nearest meteorological station should be specified. Such data should include temperature (mean, maximum and minimum in ^{o} C) and relative humidity (%). All significant changes in the weather should be noted.

3.1.2 Edaphic Data

Not required

3.2 Method, Time and Frequency of Observations

3.2.1 Method

Data on disease development and severity of infection should be recorded. The rating scale used should be relevant and well defined. . The age of the tuber should be specified.

3.2.2 Timeframe and Frequency of Observation

The timeframe and frequency of the observation should be specified: the first evaluation should be conducted just before the treatment; followed by one or several intermediary evaluations and a final evaluation.

3.3 Observations of Phytotoxicity on Crop

All positive effects of the treatment on the tubers should be noted. The tubers should be examined for the presence or absence of phytotoxic effects. In case of phytotoxicity, the symptoms (deformation, etc) should be described.

3.4 Observations of the Effects on Non-Target Organisms

3.4.1 Effects on other pest organisms

All observed effects on other pests including those not targeted, either positive or negative, should be noted.

4.0 Results

Test results should be presented in systematic and easily understandable form. They should be subjected to appropriate statistical analysis.. The report will include analysis and interpretation of the relevant data. Other stages of the evaluation will then follow. *Vide* OEPP PP 1/152 (2) standard Directive for the conduct and analysis of biological evaluation tests and OEPP PP 1/181 standard - Directive on biological evaluation of phytosanitary products for the conduct of biological evaluation tests and presentation of reports.

CHAPTER 13

SPECIFIC PROTOCOL FOR BIOLOGICAL ASSESSMENT OF PESTICIDES AGAINST PAWPAW PESTS

Introduction

This specific Protocol is developed to facilitate the conduct of experiments and comparison of the results of biological evaluation tests of new active ingredients or formulations of the pesticides designed to control pawpaw insect pests in all Member States of ECOWAS, CLISS and UEMOA.

Approval and Amendments
Initial approval by WACRP on…. PC2PS ECOWAS.

1.0 Experimental Conditions:

1.1 Target Pest, Selection of Crops and Varieties

The crop is *Carica papaya (*pawpaw) and the target pests are:
Invasive fruit fly *(Bactrocera invadens*Drew, Tsuruta and White), tobacco white fly (*Bemisia tabaci* Hennadius,) *Aleurodicus dispersus* Russel, broad mite (*Polyphagotarsonemus latus* Banks) spider mite (*Tetranychus urticae* Koch), pawpaw mealy bug (*Paracoccus marginatus* (Williams & Granard de Willink)

The insects suck the host plant and weaken it. They are found on the upper part but more often on the lower surface of the leaves. In some cases, they transmit the diseases and inject toxic substances into the plants.

1.2 Test conditions

The trial must be conducted in areas where the pest population is very high.
Culture conditions (type of soil, fertilizer application, tillage, cultivar, row spacing) should be uniform in all the trial plots and consistent with Good Agriculture Practices (GAP). The cropping history and pesticide products applied in the preceding two years must be known and recorded.

The trial should be conducted in similar or distinct agro-climatic conditions preferably in different years or growing seasons. It is important to conduct at least three independent trials in agro-ecological zones where the crop is grown

For the purpose of obtaining provisional clearance, the trial should be conducted for two years On-station /site and for one year in On-farm as spelt out below:

1.3 Experimental Procedures and Conduct of Tests

1.3.1 On-station test

Treatments The treatments consist of different rates (generally three) of the product under study, the rate of the manufacturer, ¾ and 3/2 of these rates, a reference product

known for its efficacy against the target pest and with similar mode of action to that of the product under study and an untreated control.

The experimental design should be randomised complete block.. The plot size should generally be at least ten (10) plants with 5m intervals. There should be 4 replications.

1.3.2 On-farm trials

Treatment: The treatments are generally made up of three rates. The best rate of the product derived from on-station/site tests, the reference product and an untreated control. The experimental design should be randomized design with 6 replication. Each plot will be made up of 3 lines of 15 plants each covering a surface of 180m^2

2.0 Application of Treatment

2.1 Product(s) Under Study

The product to be tested should be a formulated product and should have a specific name.

2.2 Reference Product

In view of the variability of conditions under which pesticides are used, it is necessary to include a reference product in field trials to allow meaningful evaluation of efficacy under the conditions of the trial. Satisfactory levels of efficacy will generally be met when performance is comparable to that of the reference product, which should preferably be a registered product widely accepted as satisfactory in practice. However, other considerations (e.g. manner of use, side effects, etc may arise in assessing what is a satisfactory level of efficacy.

Where the type of pesticide product or its use is new, comparison with a reference product may be impossible or inappropriate. In this case, the product under study should show a consistent well-defined benefit. The pesticide to be introduced should be able to bring and/or keep the pest population and the damage to which it gives rise below an economic or phytosanitary threshold level, where this is known.

Wherever feasible the reference product chosen should be one which has shown satisfactory results in practice and its mode of action should be the same as or similar to that of the test product. The registration authority must be consulted on issues relating to the selection of a reference product for the study. Where a there is the need to use a reference product other than the one agreed or recommended by the registration authority, a justification must be made.

2.3 Untreated Control

An untreated control is needed for all trials both on-site tests and on-station or on-farm.

2.4 Application Methods

Application should conform to good agriculture practice.

2.4.1 Method of application

The recommended method of application should be used.

2.4.2 Type of application equipment

Each application should be done with the aid of equipment that ensures uniform

distribution of the product over the entire plot or that ensures guided target treatment. Factors likely to affect efficiency of delivery such as pressure or nozzle type should be avoided. The equipment should be selected in accordance with product recommendation.

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2.4.3 Timeframe and frequency of application

The number of applications and date of each treatment should be as those indicated for the proposed usage. These will depend on the biology of the target pest. The date of the applications should be recorded. The application should start as soon as the pests appear. In such cases, four applications should be made at monthly intervals.

2.4.4 Application rate and volumes

On-station/site: The product should be tested using the recommended rate (R), by the manufacturer, ¾R and $^{3}/_{2}$ R. These rates are expected to help determine whether the rate recommended by the manufacturer is the optimal rate in terms of efficacy and economic returns under existing agro-ecological conditions.

On-farm: The optimal rate determined from on-site test is generally tested.

The rate should be expressed in kg/ha or l/ha of the formulated product and in g ai/h for the active ingredient. It may also be necessary to express this dose in g. ai/kg or g.ai/l. For formulations in powder for dust spraying, be it granulated or similar products; this should be expressed in g .ai/kg or %.

For seed treatment, the rate should be expressed in kg (or litres) of the formulated product per ton of seeds, and in g ai/kg of seeds.

2.4.5 Information on all other pesticide products

Where other pesticide products (or biological control agents) are used, they should be applied uniformly on all plots. They should not be mixed with the product under study and the reference product. The date on which the treatments were applied should be recorded. The risk of interference should be minimized.

3.0 Observations and Data Collection, Recordings and Measurements

3.1. Meteorological and Edaphic Data

3.1.1 Meteorological Data

The meteorological data before and after application must be recorded. The data obtained from the trial location or from the nearest meteorological station should be specified. Such data should include precipitations (frequency and volume) and temperature (mean, maximum and minimum in ^{o} C). All significant changes in the weather should be noted, particularly those likely to affect the test (humidity, dry spells). Precise data on all possible irrigation of the area should be indicated.

3.1.2 Edaphic data

Not mandatory

3.2 Method, time and frequency of observation

The growth stage of the crop should be recorded during each application.

3.2.1 Method of assessment

Select and tag 5 plants (1 plant in 3) in the middle row of each plot before the first application of treatment. Samples are taken on each of these plants. Subsequent samples are taken the day before treatment application to the plots.

At each sampling, the following procedure should be followed. :

- Counting of dried leaves on marked plants. s
- Counting of leaves attacked by pest to determine the extent of damage. (Low, medium, high)
- On the mid rib of the oldest fresh leaf.
- Count the number of pests and f plants attacked.

3.2.2 Timeframe and frequency of observation

At least one observation is required on the pest (1 or 3 days) before treatment in order to determine the composition of pest and the degree of infestation.

3.3 Observations of Phytotoxicity

Plants must be examined for the presence or absence of phytotoxicity on/from the leaves. Where toxicity effects are measurable, count the number of leaves showing the symptoms. The results must be indicated in absolute figures. Otherwise, phytotoxicity can be estimated based on a rating scale as against the untreated plots.

The symptoms of burning must be well described: chlorosis, faltering, and deformation.

3.4 Observations of Effects on Non-Target Organism

3.4.1 Effects on other pest

All observed effects on other pest whether positive or negative should be recorded.

3.4.2 Effects on beneficial organisms

Any positive or negative effect observed on parasitoids and predators, pollinators on adjacent or succeeding crops should be recorded. Any effect on the environment especially the effects on other fauna (both terrestrial and aquatic) should be recorded.

3.5 Qualitative and Quantitative Assessment of Yield

The percentage of marketable fruits (from about 50-100) randomly harvested should be determined. . Calculate the weight of fruits marketable per plot.

4.0 Results

The data should be analysed with the appropriate statistical tools and the results presented in a systematic and easily understandable form.. The report should include analysis and interpretation of the results.

CHAPTER 14

SPECIFIC PROTOCOL FOR ASSESSING THE BIOLOGICAL EFFICACY OF FUNGICIDES AGAINST TOMATO DISEASE.

Approval and Amendments
Initial Approval by WACRP: On… under the No PC PS ECOWAS

1.0 Experimental Conditions

1.1 Target Pathogens and Crop Varieties

The target pathogens are:

- *Fusarium spp*
- *Collectotrichum lindemuthianum* (dry rot of fruit)
- *Phytophthora spp* (wet rot of plants and fruits)
- *Alternaria solani*

The crop to be protected is tomato (*Lycopersicon esculentum)*

1.2 Trial Conditions

The trial must be carried out on a varieties in use and susceptible to the target pathogen. .The trial conditions must be favorable to the multiplication of the pathogen. The trial must be multi-locational in many parts of West Africa with different agro-climatic conditions and it should be preferably carried out during the cropping season.

1.3 Experimental Procedures and Conduct of Tests

1.3.1 On-station test

Treatments: The treatments should consist of three different rates; the manufacturer's recommended rate (R), $^{3}/4$(R) and 3/2 (R), a reference product and untreated control. The reference product should have similar mode of action to that of the test product. The experimental design should be a Randomised Complete Block with 4 replications. The plot size should generally 25 m^2 with 3 m spacing between the plots, but evaluation of damage will be done only on the middle plants. At least four (4) replications are required for each treatment.

Features of the test

- The total area will be between…… and ……..m^2
- Area of a Plot:
- Length of a Plot:
- Width of a Plot:
- Distance between Lines:

- Distance between Plots:
- Distance between blocs: Number of repetitions:
- Number of Plot per bloc:
- Total Number of Plots:

1.3.2 On-farm test

Treatment: The treatments are generally made up of three rates. The best rate of the product derived from on-site tests, the rate proposed by the manufacturer and an untreated control. The plot size should generally be … m2. Owing to the wide variations between the plots and the possibility of losing some of the treatments, at least 5 replications are required for each trial. Adequate spacing should be provided (3 m) between the plots taking into account the wind direction during treatments.

2.0 Application of Treatment

2.1 Product(s) Under Study

The product to be tested should be a formulated product and should have a specific name.

2.2 Reference Product

In view of the variability of conditions under which pesticides are used, it is necessary to include a reference product in field trials to allow meaningful evaluation of efficacy under the conditions of the trial. Satisfactory levels of the efficacy will generally be met when performance of the test product is comparable to that of the reference product, which should preferably be a registered product widely accepted as effective and satisfactory in practice. However, other considerations (e.g. manner of use, side effects, etc) may arise in assessing what is a satisfactory level of efficacy.

Where the type of pesticide product or its use is new, comparison with a reference product may be impossible or inappropriate. In this case, the product under study should show a consistent well-defined benefit. The pesticide to be introduced should be able to bring and/or keep the pest population and the damage to which it gives rise below an economic or phytosanitary threshold level, where this is known.

Wherever feasible the reference product chosen should be one which has shown satisfactory results in practice and its mode of action should be the same as or similar to that of the test product. The registration authority must be consulted on issues relating to the selection of a reference product for the study. Where there is the need to use a reference product other than the one agreed or recommended by the registration authority, a justification must be made.

2.3 Untreated Control

An untreated control is needed for all trials both on-site tests and on-station or on-farm.

2.4 Application Methods

Application should conform to GAP.

2.4.1 Method of application

The recommended method of application should be used.

2.4.2 Type of application equipment

Each application should be done with equipment that ensures uniform distribution of the product over the entire plot or that ensures guided target treatment. Factors likely to modify efficiency of delivery such as pressure or nozzle type should be avoided. The equipment should be selected in accordance with product recommendation. Proper calibration of the application equipment is essential for the correct delivery of the chemical.

2.4.3 Information on all other pesticide products

Where other pesticide products (or biological control agents) are used, they should be applied uniformly on all plots. They should not be mixed with the product under study and the reference product. The date on which the treatments were applied should be recorded. The risk of interference should be minimized.

2.4.4 Timeframe and frequency of application

The number of applications and date of each treatment should be consistent with those indicated for the proposed usage. These will depend on the stage of development of the crop and the biology of the target pest (s) such as stage of development, tolerance threshold and peak of population. The date of the applications should be recorded.

2.4.5 Application rates and volumes

On-station/site: a minimum of three rates should be tested; the manufacturer's rate, a lower and a higher rate. These rates are expected to help determine whether the rate recommended by the manufacturer is the optimal rate in terms of efficacy and economic returns under a given agro-ecological conditions.

In on-farm setting, the optimal rate determined from on-station/site test is generally tested.

The applicable rate should be expressed in g. ai/ha and in kg/ha or 1/ha of the formulated product. For liquid formulations, the concentration should be in g. ai/l. It may also be necessary to express this dose in g. ai/kg or g.ai/1. For formulations in powder for dust spraying, be it granulated or similar products; this should be expressed in g. ai/kg or %.

For seed treatment, the rate should be expressed in kg (or litres) of the formulated product per ton of seeds, and in g. ai/kg of seeds.

The actual rate applied should always be measured and any deviation from the recommended rate recorded. The frequency and date of each application must be recorded. Generally, the 1st application of the product should be made when the first symptoms are observed

2.4.6 Information on all other pesticide products

Where other pesticide products (or biological control agents) are used, they should be applied uniformly on all plots. They should not be mixed with the product under study

and the reference product. The date on which the treatments were applied should be recorded. The risk of interference should be minimized.

3.0 Observations and Data Collection, Recordings and Measurements

3.1 Meteorological Data

Days before and after product application, the meteorological data likely to influence crop development and/or that of the target pest and the action of the pesticide product should be recorded. Such data should normally include rainfall and temperature. All data should in principle, be recorded on the test site, especially for on-site test. It is sometimes difficult to obtain meteorological data in the local on-farm condition in which case, the relevant data could be obtained from the nearest meteorological station.

On the day of application, the meteorological data likely to influence the quality and persistency of the product should be recorded. Such data will normally include at least rainfall (nature and volume in mm) and temperature (mean maximum and minimum in °C). Any significant change in the day's weather should be recorded and its relation to the application should also be indicated. Throughout the test period, unusual weather conditions such as prolong dry periods, heavy rains, strong winds etc, likely to influence the outcome of the test should be recorded. Accurate data should be provided on any irrigation to be carried out on the plot.

3.1.1 Edaphic Data

The type and nature of soil, pH and organic matter should be described.

3.2 Method, Time and Frequency of Observation

The age and growth stage of the crop should be recorded during each application.

3.2.1 Methodology

Data on the density, the incidence and severity of the infection must be indicated and the rating scales defined. . The stage of the development of the crop must be indicated during each application in every plot; the first two lines are considered as borders and should not be used for assessment.

Assessment is made once a week on 20 plants selected randomly per plot. The selection follows a diagonal arrangement of the lines and is done using a square placed at each 2 meters interval.

During the assessment, the plants showing symptoms of attack on their upper parts (stems, leaves, flowers, etc) are counted. The percent efficacy (E %) of the pesticide is obtained by the following formula:

$$E\% = \frac{\text{Number of plants with symptoms}}{\text{Total number of tested plants}}$$

$$E\% = \frac{NP-NPS}{} \times 100$$

TNPT

NPS = Number of plants with symptoms

TNPT = Total number of plants tested

The pesticide is declared efficient if its efficacy is at least 90%.

3.2.2 Timeframe and frequency of observation

The time and frequency of observation should be indicated. : the first assessment must be done before the treatment application, at least one intermediary assessment conducted before the final.. There should be two more observations after the final treatment (2 or 4 weeks after the final treatment).

3.3 Observation of Direct Effects on the Crop

3.3.1. On-Farm trial

Any positive or negative effect of the treatment on the plants must be recorded. Symptoms such as shrinking, deformation and other damages on the plant caused by the pesticide should also be indicated.

3.3.2 Observations on the various phases of growth

- Vegetative
- Flowering
- Fruiting

3.3.3 Biometric measurements

- Percentage of damage on flowers
- Weight of fruits attacked
- Total yield

3.4 Observation of Effects on Non-Targeted Organisms

3.4.1 Effects on other non-targeted pests

Any effect, positive or negative, on other non-targeted pests must be indicated, especially on the flora.

3.5 Quantitative and Qualitative Assessment of the Yield Data

After the harvest, the fruits should be sorted according to the desired qualities
The yield must be determined. The method of calculations and the parameters for measuring the quality index must be indicated.

4 .0 Results /Report - Statistical Analysis

The data should be analysed with the appropriate statistical tools and the results presented in a systematic and easily understandable form.. The report should include analysis and interpretation of the results.

CHAPTER 15

PROTOCOL FOR BIOLOGICAL EVALUATION OF PESTICIDES TO CONTROL SUGAR CANE STEM BORER PEST

Introduction

This specific protocol has been prepared to facilitate the conduct of field trials and effective comparison of test results in all Member States of ECOWAS, CILSS and UEMOA.

This section defines the general principles for biological evaluation of new active ingredients or formulations of insecticides designed to control sugar cane stem borer pest..

Approvals and Amendments

Initial Approval by WAPRC: ..... July 2008 Reference No. PC 2 PS 04_ECOWAS.

1.0 Experimental Conditions

1.1 Target Pest, Selection of Crops and Varieties

The target stem borer pest species should be specified. The major species associated with the sugar cane is *Eldana saccharina* Walker (*Lepidoptera-Noctuidae*). Other species could also attack the plant during the growing stage. The most important is *Sesamia calamistis* Hampson (*Lepidoptera-Noctuidae*).

It is important to take into consideration the biology and ecology of each species used in the conduct of the experiment. Thus the test should be conducted when there is high pest pressure of the pest concerned or the borer species, to ensure that the high infestation period coincide with the various stages of development. The test should take into account the different species and their stages of development.
The variety of sugar cane (*Saccharum oficinarum*), of which numerous varieties are cultivated should be susceptible to the target pest borer insects.
For treatment of stem cuttings, care should be taken to ensure their viability.

1.2 Test Conditions

1.2.1 Test Conditions

The test should be carried out in areas where the pest pressure is generally high. The cropping condition (type of soil, fertilizer application, tillage, cultivar, row spacing) should be uniform in all the test plots and consistent with good agriculture practices. The cropping history and pesticide products applied in the preceding two years must be known and recorded.

The test should be conducted in similar or distinct agro-climatic conditions preferably in different years or growing seasons. It is important to conduct at least three independent tests in each agro-ecological zone (Sahel, Sudan and guinea etc). For the purpose of obtaining provisional registration, the test should be conducted for two years on-site and for one year in on-farm as spelt out hereunder:

Year one: on-site test. This should be conclusive with reliable results.

Year two: on-site test/on-farm, taking the agro ecological zoning into account.

For the purpose of registration, two additional tests in on-farm conditions are necessary. It proposed that the tests be conducted by different institutions or individuals accredited by WAPRC.

1.3 Experimental Procedures and Conduct of Tests

1.3.1 On-site test

Treatments: The treatments consist of different rates (generally three) of the product under study, a reference product and an untreated control. The experimental design should be randomised complete block design. The plot size should generally be 25-100m^2. At the least 5 replications are required for each treatment.
The plots being treated should be arranged in a way that avoids contamination between the various objects under comparison. To this end, sufficient space (2-3m) should be provided between the elementary plots within each block and (2-3m) between the blocks.

1.3.2 On-farm test

Treatment: The treatments are generally made up of three rates. The best rate of the product derived from on-site tests, the rate proposed by the manufacturer and an untreated control. The plot size should generally be 100-500m^2. Owing to the wide variations between the plots and the possibility of losing some of the treatments, at least 8 replications (8 peasant farms) are required for every test. Enough space should be provided (3-5m) between the plots taking into account the wind direction during treatments.

2.0 Application of Treatment

2.1 Product(s) Under Study

The product to be tested should be a formulated product and should have a specific name.

2.2 Reference Product

In view of the variability of conditions under which pesticides are used, it is necessary to include a reference product in field trials to allow meaningful evaluation of efficacy under the conditions of the trial. Satisfactory levels of efficacy will generally be met when performance is comparable to that of the reference product, which should preferably be a registered product widely accepted as satisfactory in practice. However, other considerations (e.g. manner of use, side effects, etc may arise in assessing what is a satisfactory level of efficacy.

Where the type of pesticide product or its use is new, comparison with a reference product may be impossible or inappropriate. In this case, the product under study should show a consistent well-defined benefit. The pesticide to be introduced should be able to bring and/or keep the pest population and the damage to which it gives rise below an economic or phytosanitary threshold level, where this is known.

Wherever feasible the reference product chosen should be one which has shown satisfactory results in practice and its mode of action should be the same as or similar to that of the test product. The registration authority must be consulted on issues relating to the selection of a reference product for the study. Where a there is the need to use a reference product other than the one agreed or recommended by the registration authority, a justification must be made.

2.3 Untreated Control

An untreated control is needed for on-site tests and also for on-farm. Test should be conducted under local farming conditions.

2.4 Application Methods

Application should conform to good agriculture practice.

2.4.1 Method of application

The recommended method of application should be used.

2.4.2 Type of application equipment

Each application should be done with the aid of equipment that ensures uniform distribution of the product over the entire plot or that ensures guided target treatment. Factors likely to modify efficiency of delivery such as pressure or nozzle type should be avoided. The equipment should be selected in accordance with product recommendation.

For stem cuttings, treatment should be carried out with equipment which ensures uniform distribution of the product in accordance with recommended practice.

2.4.3 Timeframe and frequency of application

The number of applications and date of each treatment should be as those indicated for the proposed usage. These will depend on the biology of the target pest. The date of the applications should be recorded.

2.4.4 Application rates and volumes

On-site: a minimum of three rates should be tested, the manufacturers rate, a lower rate and a higher rate. The exact choice of rate is expected to help determine if the rate proposed by the manufacturer is the optimal rate in terms of effectiveness and economic returns under humid/Sahel conditions.
Local farming setting: the optimal rate ascertained from the on-site test is generally tested.
The applicable rate should be expressed in g ai/ha and in kg/ha or l/h for the formulated product. For liquid formulations, the concentration should be in g ai/l.

For formulations in powder for dust spraying, be it granulated or similar products; this should be expressed in g ai/kg or %.

The actual rate applied should always be measured and any deviation from the recommended rate recorded.

2.4.5 Information on other pesticide products

Where other pesticide products (or biological control agents) are used, they should be applied uniformly on all plots. They should not be mixed with the product under study and the reference product. The date on which the treatments were applied should be recorded. The risk of interference should be minimized.

3.0 Observations and Data Collection, Recordings and Measurements

3.1 Meteorological and Edaphic Data

Days before and after product application, the meteorological data likely to influence crop development and/or that of the target pest and the action of the pesticide product should be

recorded. Such data should normally include rainfall and temperature. All data should in principle, be recorded on the test site, especially for on-site test. It sometimes difficult to obtain meteorological data in the local on-farm condition in which case, the relevant data could be obtained from the nearest meteorological station.

On the day of application, the meteorological data likely to influence the quality and persistency of the product should be recorded. Such data will normally include at least rainfall (nature and volume in mm) and temperature (mean maximum and minimum in ^{o}C) any significant change in the days weather should be recorded and in relation to the application indicated.

Throughout the test period, prolong dry periods, heavy rains, sandy winds etc, likely to influence the outcome of the test should be recorded. Accurate data should be provided on any irrigation to be carried out on the plot.

3.1.1 Edaphic data

The Edaphic data to be gathered include soil type (international standards to be specified), pH, organic matter content, soil moisture, and soil structure and texture and soil fertility status.

Where experiments are carried out on plants cultivated in humus or any other artificial media these should be described in detail. The description should include the irrigation system in which the substrates are kept.

3.2 Method, Observation and Data Collection

The growth stage of the crop should be recorded during each application and observation.

3.2.1 Method

The method chosen should lend itself to statistical analysis to determine the efficacy of the product. The data should be collected on 20 plants per plot from the middle rows on the ff: the healthy tillers, infested tillers (deadheart), stem with insects entry or exit openings and healthy stem. The larvae and pupae populations density should be observed outside the middle rows.20 plants randomly selected from the outer row should be collected and dissected.

3.2.2. Timeframe and frequency of observation

At least one observation in required on pest infestation and damage (generally 1-3 days) before the first treatment during the growing season. A sample of 20 plants per plot should be dissected before treatment to clarify the composition of the pest complex or estimate the degree of infestation. The observation should be carried out during the vegetative and reproductive stages. The frequency should be once a week. The observations should also take into account the level of the pest populations, the stages of pest development as well as the speed of performance and persistency of the product.

3.3 Observations of Phytotoxicity

Phytotoxicity should be examined and all positive or negative effects recorded.

3.4 Observations of Effects on Non-target Organism

3.4.1 Effects on other pest

All observed effects on other pest be it positive or negative should be recorded.

3.4.2 Effects on beneficial organisms

Any positive or negative effect observed on parasitoids and predators, pollinators on adjacent or succeeding crops should be recorded. Any effect on the environment especially the effects on other fauna (both terrestrial and aquatic) should be described.

3.5 Quantitative and Qualitative Evaluation of Harvested Crop

Although it is recognized that crop yield depends on several factors, quantitative and/or qualitative assessment of harvested crop is required. Thus the cane yield should be provided (tones/ha)

4.0 Results

Test results should be presented in systematic and easily understandable form. They should be subjected to statistical analysis using pre-established methods. The report will include analysis and interpretation of the relevant data. Other stages of the evaluation will then follow. *Vide* OEPP PP 1/152 (2) standard Directive for the conduct and analysis of biological evaluation tests and OEPP PP 1/181 standard - Directive on biological evaluation of phytosanitary products for the conduct of biological evaluation tests and presentation of reports.

CHAPTER 16

PROTOCOL FOR BIOLOGICAL EVALUATION OF PESTICIDES TO CONTROL SORGHUM, MILLET AND MAIZE STEM BORERS

Introduction

This specific Protocol has been prepared to facilitate the conduct of field trials and effective of biological evaluation tests on cereals (sorghum, millet and maize) stem borer insects in all Member States of ECOWAS, CILSS and UEMOA.

It describes the principles for the for the conduct of biological evaluation test on new active ingredients or insecticide formulations designed to control sorghum, millet and maize stem borer insects (Lepidoptera stem borers and shoot flies)

Approvals and Amendments

Initial Approval by WAPRC: ..... July 2008, Reference No. PC2 PS 05_ECOWAS

1.0 Experimental Conditions

1.1 Target Pest, Selection of Crops and Varieties

The target stem borer species should be specified. The major species associated with the three cereals are basically of two orders: Lepidoptera and dipteral. Their significance varies according to the crop and prevailing conditions (climatic and soil conditions as well as cropping practices).

The stem borer Lepidoptera species associated with the three cereals are as follows:

- *Busseola fusca* fuller (Noctuidae)
- *Sesamia calamistis* Hampson(Noctuidae)
- *Eldana saccharina* Walker (Pyralidae)
- *Coniesta ignefusalis* Hampson(Pyralidae) and
- *Chilo diffusilineus* de joannis(Pyralidae)

The dipteral species (shoot flies) are as follows:

- *Atherigona soccata* Rondani(Muscidae) and
- *Atherigona sp*

It is important to take into consideration the biology and ecology of each species used in the conduct of the experiment. Thus the test should be conducted when there is high pest pressure of the pest concerned or the borer species, to ensure that the high infestation period coincide with the stages.
The test should take into account the different species and their stages of development.

The variety of sorghum (*sorghum bicolor*), maize (*zea mais*) and millet (*Pennisetum glaucum*) used should be susceptible to the target pest borer insects.
For seed treatment, the rate of germination of the variety used should be determined.

1.2 Test Conditions

The test should be carried out in areas where the pest pressure is generally high. The cropping condition (type of soil, fertilizer application, tillage, cultivar, row spacing) should be uniform in all the test plots and consistent with good agriculture practices. The cropping history and pesticide products applied in the preceding two years must be known and recorded.

The test should be conducted in similar or distinct agro-climatic conditions preferably in different years or growing seasons. It is important to conduct at least three independent tests in each agro-ecological zone (Sahel, Sudan and guinea etc). For the purpose of obtaining provisional registration, the test should be conducted for two years on-site and for one year in on-farm as spelt out hereunder:

Year one: on-site test. This should be conclusive with reliable results.

Year two: on-site test/on-farm, taking the agro ecological zoning into account.

For the purpose of registration, two additional tests in on-farm conditions are necessary. It proposed that the tests be conducted by different institutions or individuals accredited by WAPRC.

1.3 Experimental Procedures and Conduct of Tests

1.3.1 On-site test

Treatments: The treatments consist of different rates (generally three) of the product under study, a reference product and an untreated control. The experimental design should be randomised complete block design. The plot size should generally be 50-100m^2. At the least 4 replications are required for each treatment.
The plots being treated should be arranged in a way that avoids contamination between the various objects under comparison. To this end, sufficient space (1-2m) should be provided between the elementary plots within each bloc and (2-3m) between the blocks.

1.3.2 on-farm test

Treatment: The treatments are generally made up of three rates. The best rate of the product derived from on-site tests, the rate proposed by the manufacturer and an untreated control. The plot size should generally be 200-300m^2. Provision should be made for 5-8 replications localised preferably in the farms belonging to the local farmers that have shown an interest in the approach. Owing to the wide variations between the plots and the possibility of losing some of the treatments, at least 8 replications (8 peasant farms) are required for every test. Enough space should be provided (1-2m) between the plots taking into account the wind direction during treatments.

2.0 Application of Treatment

2.1 Product(s) Under Study

The product to be tested should be a formulated product and should have a specific name.

2.2 Reference Product

In view of the variability of conditions under which pesticides are used, it is necessary to include a reference product in field trials to allow meaningful evaluation of efficacy under the conditions of the trial. Satisfactory levels of efficacy will generally be met when performance is comparable to that of the reference product, which should preferably be a registered product widely accepted as satisfactory in practice. However, other considerations (e.g. manner of use, side effects, etc may arise in assessing what is a satisfactory level of efficacy.

Where the type of pesticide product or its use is new, comparison with a reference product may be impossible or inappropriate. In this case, the product under study should show a consistent well-defined benefit. The pesticide to be introduced should be able to bring and/or keep the pest population and the damage to which it gives rise below an economic or phytosanitary threshold level, where this is known.

Wherever feasible the reference product chosen should be one which has shown satisfactory results in practice and its mode of action should be the same as or similar to that of the test product. The registration authority must be consulted on issues relating to the selection of a reference product for the study. Where a there is the need to use a reference product other than the one agreed or recommended by the registration authority, a justification must be made.

2.3 Untreated Control

An untreated control is needed for on-site tests and also for on-farm. Test should be conducted under local farming conditions.

2.4 Application Methods

Application should conform to good agriculture practice.

2.4.1 Method of application

The recommended method of application should be used.

2.4.2 Type of application equipment

Each application should be done with the aid of equipment that ensures uniform distribution of the product over the entire plot or that ensures guided target treatment. Factors likely to modify efficiency of delivery such as pressure or nozzle type should be avoided. The equipment should be selected in accordance with product recommendation.

Seeds should be treated with equipment that allows for uniform distribution of the product in accordance with recommended practice.

2.4.3 Timeframe and frequency of application

The number of applications and date of each treatment should be as those indicated for the proposed usage. These will depend on the biology of the target pest. The date of the applications should be recorded.

2.4.4 Application rates and volumes

On-site: a minimum of three rates should be tested, the manufacturers rate, a lower rate and a higher rate. The exact choice of rate is expected to help determine if the rate proposed by the manufacturer is the optimal rate in terms of effectiveness and economic returns under humid/Sahel conditions.
Local farming setting: the optimal rate ascertained from the on-site test is generally tested.
The applicable rate should be expressed in g ai/ha and in kg/ha or l/h for the formulated product. For liquid formulations, the concentration should be in g/l.
For seed treatment, the rate should be expressed in kg (or litres) of the formulated product per ton of seeds, and in g ai/kg of seeds.

The actual rate applied should always be measured and any deviation from the recommended rate recorded.

2.4.5 Information on other pesticide products

Where other pesticide products (or biological control agents) are used, they should be applied uniformly on all plots. They should not be mixed with the product under study and the reference product. The date on which the treatments were applied should be recorded. The risk of interference should be minimized.

3.0 Observations and Data Collection, Recordings and Measurements

3.1 Meteorological and Edaphic Data

Days before and after product application, the meteorological data likely to influence crop development and/or that of the target pest and the action of the pesticide product should be recorded. Such data should normally include rainfall and temperature. All data should in principle, be recorded on the test site, especially for on-site test. It sometimes difficult to obtain meteorological data in the local on-farm condition in which case, the relevant data could be obtained from the nearest meteorological station.

On the day of application, the meteorological data likely to influence the quality and persistency of the product should be recorded. Such data will normally include at least rainfall (nature and volume in mm) and temperature (mean maximum and minimum in ^{o}C) any significant change in the days weather should be recorded and in relation to the application indicated.

Throughout the test period, prolong dry periods, heavy rains, sandy winds etc, likely to influence the outcome of the test should be recorded. Accurate data should be provided on any irrigation to be carried out on the plot.

3.1.1 Edaphic data

The Edaphic data to be gathered include soil type (international standards to be specified), pH, organic matter content, soil moisture, and soil structure and texture and soil fertility status.

Where experiments are carried out on plants cultivated in humus or any other artificial media these should be described in detail. The description should include the irrigation system in which the substrates are kept.

3.2 Method, Observation and Data Collection

The growth stage of the crop and number of days after seeding or emergence should be recorded during each application and observation.

3.2.1 Method

The sampling or observation method depends on the borer insect pest under investigation. The method chosen should lend itself to statistical analysis to determine the efficacy of the product. With regard to damage assessment, 'deadheart' symptoms or scorching should be recorded generally 14, 25 and 42 days after germination. At maturity data should be taken on the following: broken stalk and panicles (sorghum and millet), infestation of ears (maize) from the 100 plants randomly selected per plot. At harvest, 50-100 stems randomly selected per plot and dissected to assess stem borer damage. The number of the larvae and pupae of the various species should also be observed and recorded.

3.2.2. Timeframe and frequency of observation

After germination at least one observation in required on pest infestation and damage (generally 1-3 days) before the first treatment during the growing season.

3.3 Observation of Phytotoxicity on the Crop

Phytotoxicity should be examined and all positive or negative effects recorded.

3.4 Observations of Effects on Non-target Organism

3.4.1 Effects on other pest

All observed effects on other pest are it positive or negative should be recorded.

3.4.2 Effects on beneficial organisms

Any positive or negative effect observed on parasitoids and predators, pollinators on adjacent or succeeding crops should be recorded. Any effect on the environment especially the effects on other fauna (both terrestrial and aquatic) should be described.

3.5 Quantitative and Qualitative Evaluation of Harvested Crop

Although it is recognized that crop yield depends on several factors, quantitative and/or qualitative assessment of harvested crop is required. Depending on the crop, such evaluation could include yield assessment such as the number of panicles or ears/m2, average weight/panicle or ears, average weight of grains/panicle or ears and weight of 1000 grains) and finally calculation or yield per hectare (adjusted to 14% moisture content)

4.0 Results

Test results should be presented in systematic and easily understandable form. They should be subjected to statistical analysis using pre-established methods. The report will include analysis and interpretation of the relevant data obtained by monitoring all stages of the biological evaluation.

CHAPTER 17

PROTOCOL FOR BIOLOGICAL EVALUATION OF PESTICIDES TO CONTROL RICE STEM BORER

Introduction

This Framework Protocol has been prepared to facilitate the conduct of field trials and effective of biological evaluation tests on rice stem borer insects in all Member States of ECOWAS, CILSS and UEMOA.

It describes the principles for the for the conduct of biological evaluation test on new active ingredients or insecticide formulations designed to control rice stem borer.

Approvals and Amendments

Initial Approval by WAPRC: ..... July 2008, Reference No. PC2 PS 06_ECOWAS

1.0 Experimental Conditions

1.1 Target Pest, Selection of Crops and Varieties

The stem borer pest should be described. The major species belong to two main orders.

Lepidoptera: this order is made up of the following species

- *Maliiarpha separatella* Ragonot(*Pyralidae*)
- *Chilo zacconius* Bleszynski (*Pyralidae*)
- *Chilo diffusilineus* J. de Joanis(*Pyralidae)*
- *Chilo alleniellus* Strand(*Pyralidae)*
- *Scirphaga melanoclista* Meyrick(*Pyralidae)*
- *Scirphaga subumbrosa* Meyrick(*Pyralidae)*
- *Sesamia calamistis* Hampson(*Noctuidae)*
- *Sesamia spp(Noctuidae)*

Diptera:
There are two main species that infest rice namely:

- *Diopsis apicalis* Dalman*(Diopsidae)* and
- *Diopsis thoracica* Westwood*(Diopsidae)*

The biology of the species should be taken into consideration in the timing of the experiment. This mean in particular, conducting the test at the periods when crops are likely to be subject to significant pest infestation. The tests should be carried out on the organisms and stages of development of the organism so tested should be indicated in the proposed usage.

The rice variety (*oryza sativa or oryza glaberrima)* should be susceptible to stem borer insects.
For seed treatment, the rate of germination of the variety used should be determined.

1.2 Test Conditions

1.2.1 Test Conditions

The test should be carried out in areas where the pest pressure is generally high. The cropping condition (type of soil, fertilizer application, tillage, cultivar, row spacing) should be uniform in all the test plots and consistent with good agriculture practices. The cropping history and pesticide products applied in the preceding two years must be known and recorded.

The test should be conducted in similar or distinct agro-climatic conditions preferably in different years or growing seasons. It is important to conduct at least three independent tests in each agro-ecological zone (Sahel, Sudan and guinea etc). For the purpose of obtaining provisional registration, the test should be conducted for two years on-site and for one year in on-farm as spelt out hereunder:

Year one: on-site test this should be conclusive with reliable results.

Year two: on-site test and on-farm, taking the agro ecological zoning into account.

For the purpose of registration, two additional tests in on-farm conditions are necessary. It proposed that the tests be conducted by different institutions or individuals accredited by WAPRC.

1.3 Experimental Procedures and Conduct of Tests

1.3.1 On-site test

Treatments: The treatments consist of different rates (generally three) of the product under study, a reference product and an untreated control. The experimental design should be randomised complete block design. The plot size should generally be 25-100m^2. At the least 5 replications are required for each test.
The plots being treated should be arranged in a way that avoids contamination between the various objects under comparison. To this end, sufficient space (2-3m) should be provided between the elementary plots within each block and (2-3m) between the blocks.

1.3.2 on-farm test

Treatment: The treatments are generally made up of three rates. The best rate of the product derived from on-site tests, the rate proposed by the manufacturer and an untreated control. The plot size should generally be 50-500m^2. Owing to the wide variations between the plots and the possibility of losing some of the treatments, at least 8 replications (8 peasant farms) are required for every test. The plots should be arranged in a way to avoid cross contamination of treatments. This can be done by providing sufficient space (2-3m) between the plots and taking into consideration the wind direction during treatment or by using protective screens between the plots.

2.0 Application of Treatment

2.1 Product(s) Under Study

The product to be tested should be a formulated product and should have a specific name.

2.2 Reference Product

In view of the variability of conditions under which pesticides are used, it is necessary to include a reference product in field trials to allow meaningful evaluation of efficacy under the

conditions of the trial. Satisfactory levels of efficacy will generally be met when performance is comparable to that of the reference product, which should preferably be a registered product widely accepted as satisfactory in practice. However, other considerations (e.g. manner of use, side effects, etc may arise in assessing what is a satisfactory level of efficacy.

Where the type of pesticide product or its use is new, comparison with a reference product may be impossible or inappropriate. In this case, the product under study should show a consistent well-defined benefit. The pesticide to be introduced should be able to bring and/or keep the pest population and the damage to which it gives rise below an economic or phytosanitary threshold level, where this is known.

Wherever feasible the reference product chosen should be one which has shown satisfactory results in practice and its mode of action should be the same as or similar to that of the test product. The registration authority must be consulted on issues relating to the selection of a reference product for the study. Where a there is the need to use a reference product other than the one agreed or recommended by the registration authority, a justification must be made.

2.3 Untreated Control

An untreated control is needed for on-site tests and also for on-farm. Test should be conducted under local farming conditions.

2.4 Application Methods

Application should conform to good agriculture practice.

2.4.1 Method of application

The recommended method of application should be used.

2.4.2 Type of application equipment

Each application should be done with the aid of equipment that ensures uniform distribution of the product over the entire plot or that ensures guided target treatment. Factors likely to efficiency of delivery such as pressure or nozzle type should be avoided. The equipment should be selected in accordance with product recommendation.

Seeds should be treated with equipment that allows for uniform distribution of the product in accordance with recommended practice.

2.4.3 Timeframe and frequency of application

The number of applications and date of each treatment should be as those indicated for the proposed usage. These will depend on the biology of the target pest. The date of the applications should be recorded.

2.4.4 Application rates and volumes

On-site: a minimum of three rates should be tested, the manufacturers rate, a lower rate and a higher rate. The exact choice of rate is expected to help determine if the rate proposed by the manufacturer is the optimal rate in terms of effectiveness and economic returns under humid/Sahel conditions.
Local farming setting: the optimal rate ascertained from the on-site test is generally tested.
The applicable rate should be expressed in g ai/ha and in kg/ha or l/h for the formulated product. For liquid formulations, the concentration should be in g/l.

For seed treatment, the rate should be expressed in kg (or litres) of the formulated product per ton of seeds, and in g ai/kg of seeds.

The actual rate applied should always be measured and any deviation from the recommended rate recorded.

2.4.5 Information on other pesticide products

Where other pesticide products (or biological control agents) are used, they should be applied uniformly on all plots. They should not be mixed with the product under study and the reference product. The date on which the treatments were applied should be recorded. The risk of interference should be minimized.

3.0 Observations and Data Collection, Recordings and Measurements

3.1 Meteorological and Edaphic Data

Days before and after product application, the meteorological data likely to influence crop development and/or that of the target pest and the action of the pesticide product should be recorded. Such data should normally include rainfall and temperature. All data should in principle, be recorded on the test site, especially for on-site test. It sometimes difficult to obtain meteorological data in the local on-farm condition in which case, the relevant data could be obtained from the nearest meteorological station.

On the day of application, the meteorological data likely to influence the quality and persistency of the product should be recorded. Such data will normally include at least rainfall (nature and volume in mm) and temperature (mean maximum and minimum in ^{o}C) any significant change in the days weather should be recorded and in relation to the application indicated.

Throughout the test period, prolong dry periods, heavy rains, sandy winds etc, likely to influence the outcome of the test should be recorded. Accurate data should be provided on any irrigation to be carried out on the plot.

3.1.1 Edaphic data

The Edaphic data to be gathered include soil type (international standards to be specified), pH, organic matter content, soil moisture, and soil structure and texture and soil fertility status.

Where experiments are carried out on plants cultivated in humus or any other artificial media these should be described in detail. The description should include the irrigation system in which the substrates are kept.

3.2 Method, Observation and Data Collection

The growth stage of the crop should be recorded during each application and observation.

3.2.1 Method

The sampling or observation method depends on the pest under investigation. The method chosen should lend itself to statistical analysis to determine the efficacy of the product.

In the case of Lepidoptera, deadheart and white panicles are observed on the 100 plant randomly selected in the relevant plot. At harvest, the yield data should be assessed.
For Diptera, observations should focus essentially on deadheart during the growing stage. The yield should be assessed at harvest. The larvae and pupae population density should be observed outside the middle rows. To this end, dissection of the 50 stem randomly selected from the outer rows should be dissected.

3.2.2. Timeframe and frequency of observation

After germination at least one observation in required on pest infestation and damage (generally 1-3 days) before the first treatment during the growing season. A specimen of 50 plats per plot could be dissected before treatment to clarify the composition of pest complex or to estimate the degree of insect parasitism.
The observations should be carried out during the growing and reproduction stages at intervals of one week. The observation should take into account the level of pest population, their stage of development, speed of product action and product persistency.

3.3 Observation of Phytotoxicity on the Crop

Phytotoxicity should be examined and all positive or negative effects recorded.

3.4 Observations of Effects on Non-target Organism

3.4.1 Effects on other pest

All observed effects on other pest are it positive or negative should be recorded.

3.4.2 Effects on beneficial organisms

Any positive or negative effect observed on parasitoids and predators, pollinators on adjacent or succeeding crops should be recorded. Any effect on the environment especially the effects on other fauna (both terrestrial and aquatic) should be described.

3.5 Quantitative and Qualitative Evaluation of Harvested Crop

Although it is recognized that crop yield depends on several factors, quantitative and/or qualitative assessment of harvested crop is required. Depending on the crop, such evaluation could include yield assessment such as the number of panicles or ears/m2, average weight/panicle or ears, average weight of grains/panicle or ears and weight of 1000 grains) and finally calculation or yield per hectare (adjusted to 14% moisture content)

4.0 Results

Test results should be presented in systematic and easily understandable form. They should be subjected to statistical analysis using pre-established methods. The report will include analysis and interpretation of the relevant data obtained by monitoring all stages of the biological evaluation.

CHAPTER 18

PROTOCOL FOR BIOLOGICAL EVALUATION OF PESTICIDES TO CONTROL LEGUME POD FEEDING INSECTS.

Introduction

This specific Protocol is developed to facilitate the conduct of experiments and comparison of the results of biological evaluation tests of new active ingredients or formulations of the pesticides designed to control legume pod feeding insects in all Member States of ECOWAS, CLISS and UEMOA.

Approvals and Amendments

Initial Approval by WAPRC: … July 2008, Reference No. PC 2 PS 15_ ECOWAS

1.0 Experimental Conditions

1.1 Target Pest, Selection of Crops and Varieties

The insects concerned are as follows:
Pod sucking bugs: *Clavigalla tomentosicollis, Anoplocnemis curvipes, Riptortus dentipes, Mirperus jaculus, Nezara viridula*
Pod borer worms: Maruca vitrata andCydia ptychora.

The biology of the species should be taken into consideration in the timing of the experiment. This mean in particular, conducting the test at the periods when crops are likely to be subject to significant pest infestation. The tests should be carried out on the organisms and stages of development of the organism so tested should be indicated in the proposed usage.
The relevant plant is niébé or cowpea (*Vigna unguiculata*) and runner beans (*Phaseolus vulgaris*)

1.2 Test Conditions

The tests should be carried out in areas where the target pest(bug and the borer worm) pressure is generally high. The cropping condition (type of soil, fertilizer application, tillage. Cultivar, row spacing, for example) should be uniform in all the tests plots and consistent with local agricultural practices. The cropping history and the pesticide products applied in the preceding two years must be known.

The test should be conducted in similar or distinct agro-climatic conditions preferably in different years or growing seasons. It is important to conduct at least three independent tests in each agro-ecological zone (Sahel, Sudan and guinea etc).
For the purpose of obtaining provisional registration, the test should be conducted for two years on-site and for one year in on-farm as spelt out hereunder:

Year one: on-station test. Trial must be conclusive and reliable.
Year two: on-station and on-farm test at different agro ecological zones.

For the purpose of registration, two additional tests in on-farm conditions are necessary. It proposed that the tests be conducted by different institutions or individuals accredited by WAPRC.

1.3 Experimental Procedures and Conduct of Tests

1.3.1 On-site test

Treatments: The treatment consists of three rates of the product under study including those of manufacturer, ¾ and $^3/_2$ of this rate; a reference product registered and known to be effective against bugs and borer worms, mode of action of which is sufficiently close to that of the product under consideration; and an untreated control.. The experimental design should be randomised complete block design the plot size should generally be $25m^2$. The number of replications is 4.

1.3.2 On-farm test:

Treatments: The treatments consist of different rates (generally three) of the product under study, a reference product and an untreated control. The experimental design should be randomised complete block design. The plot size should generally $100m^2$. The number of replications is 5.

2.0 Application of Treatment

2.1 Product(s) Under Study

The product to be tested should be a formulated product and should have a specific name.

2.2 Reference Product

In view of the variability of conditions under which pesticides are used, it is necessary to include a reference product in field trials to allow meaningful evaluation of efficacy under the conditions of the trial. Satisfactory levels of efficacy will generally be met when performance is comparable to that of the reference product, which should preferably be a registered product widely accepted as satisfactory in practice. However, other considerations (e.g. manner of use, side effects, etc may arise in assessing what is a satisfactory level of efficacy.

Where the type of pesticide product or its use is new, comparison with a reference product may be impossible or inappropriate. In this case, the product under study should show a consistent well-defined benefit. The pesticide to be introduced should be able to bring and/or keep the pest population and the damage to which it gives rise below an economic or phyto-sanitary threshold level, where this is known.

Wherever feasible the reference product chosen should be one which has shown satisfactory results in practice and its mode of action should be the same as or similar to that of the test product. The registration authority must be consulted on issues relating to the selection of a reference product for the study. Where a there is the need to use a reference product other than the one agreed or recommended by the registration authority, a justification must be made.

2.3 Untreated Control

An untreated control is needed for on-site tests as well as test conducted under local farming conditions.

2.4 Application Methods

Applications should conform to good agricultural practices.

2.4.1 Method of *Application*

The method of application should be that which is recommended and should be specified. (Example: spraying, vaporization, fumigation, dust application and/or incorporation of granules into the soil).

2.4.2 Type of *Application Equipment*

The type of application equipment used should be specified. In all cases, the application should be done with the aid of equipment that ensures uniform distribution of the product over the entire plot or that which ensures guided on-target treatment. Factors likely to affect the efficiency of delivery (pressure and type of nozzle) should be selected in accordance with product recommendations. Seeds should be treated with equipment that allows for uniform distribution of the product in accordance with recommended practice.

2.4.3 Timeframe and Frequency of *Application*

The number of applications and the date of each application should be as recommended for the proposed usage. These will depend on the objective of the evaluation and should be consistent with the stage of development of the crop and the target organism.

2.4.4 Application rate and volumes

The product should be tested using the recommended rate(R). With respect to efficacy tests, at least two (2) supplementary rates should be used, a lower and a higher rate. The exact choice of rate is expected to help determine whether the rate recommended by the manufacturer is the optimal rate in terms of efficacy and economic returns under a given agro-ecological conditions.
In on-farm setting, the optimal rate determined from on-site test is generally tested.
The rate should be expressed in kg/ha or l/ha of the formulated product and in g ai/h for the active ingredient. It may also be necessary to express this dose in g ai/kg or g.ai/l. for formulations in powder for dust spraying, be it granulated or similar products; this should be expressed in g ai/kg or %.
The actual rate applied should always be measured and any deviation from the recommended rate recorded.

2.4.5 Information on all other pesticide products

Where other pesticide products (or biological control agents) are used, they should be applied uniformly on all plots. They should not be mixed with the product under study and the

reference product. The date on which the treatments were applied should be recorded. The risk of interference should be minimized.

3.0 Observations and Data Collection, Recordings and Measurements

3.1 Meteorological Data

Days before and after product application, the meteorological data likely to influence crop development and/or that of the target pest and the action of the pesticide product should be recorded. Such data should normally include rainfall and temperature. All data should in principle, be recorded on the test site, especially for on-site test. It sometimes difficult to obtain meteorological data in the local on-farm condition in which case, the relevant data could be obtained from the nearest meteorological station.
On the day of application, the meteorological data likely to influence the quality and persistency of the product should be recorded. Such data will normally include at least rainfall (nature and volume in mm) and temperature (mean maximum and minimum in ^{o}C) any significant change in the days weather should be recorded and in relation to the application indicated.
Throughout the test period, prolong dry periods, heavy rains, sandy winds etc, likely to influence the outcome of the test should be recorded. Accurate data should be provided on any irrigation to be carried out on the plot.

3.1.1 Edaphic data

Not required.

3.2 Method, Time and Frequency of Observation

The growth stage of the crop should be recorded during each application.

3.2.1 Method

Prior to application of the product, the number of live insects present in the plot should be estimated. The number of insects on 50 plants per plot should be counted and recorded. The damage on flower buds and pods per plot should be assessed.

3.2.2 Timeframe and Frequency of observation

The treatment should be done at regular intervals; for example, every 7 or 14 days from 1st day of application up to the harvest time.
1st Counting: 2 days after application: count the lice, as well as live pod borer larvae.
2nd Counting: 7 days after application: count the lice, as well as live pod borer larvae.
3rd Counting: 14 days after application: count the lice, as well as live pod borer larvae.
At harvest, randomly select 50 pods per plot and count the damage pods.
In cases where several applications have been carried out, the counting should take place prior to each application, and then 2, 7 and 14 days thereafter.

3.3 Observations of Phytotoxicity

Possible phytotoxic effects (or product traces) on the crop should be examined and all positive effects, noted. The nature and magnitude of these phenomena should be described; and where there has been no effect, this fact should also be noted. Where toxicity effects are measurable, the number of leaves showing the symptoms should be noted.
In the contrary event, the phytotoxicity damage may be estimated, using a notation scale in case of untreated parcels.
In all cases, symptoms of scorching should be thoroughly described: chlorosis, stunting, wilting, deformation, etc.

3.4 Observations of Effects on Non-Target Organism

3.4.1 Effects on other pest

All observed effects on other pest whether positive or negative should be recorded.

3.4.2 Effects on beneficial organisms

Any positive or negative effect observed on parasitoids and predators, pollinators on adjacent or succeeding crops should be recorded. Any effect on the environment especially the effects on other fauna (both terrestrial and aquatic) should be recorded.

3.5 Quantitative and Qualitative Evaluation of Harvested Crop

The yield is expressed in kg/ha.

4.0 Results

Test results should be presented in systematic and easily understandable form. They should be subjected to statistical analysis using pre-established methods. The report should include analysis and interpretation of the relevant data. Other stages of the evaluation will then follow. *Vide* OEPP PP 1/152 (2) standard - . Directive for the conduct and analysis of biological evaluation tests and OEPP PP 1/181 standard - Directive on biological evaluation of phytosanitary products for the conduct of biological evaluation tests and presentation of reports.

CHAPTER 19

PROTOCOL FOR BIOLOGICAL EVALUATION OF INSECTICIDES AGAINST FRUIT FLIES.

Introduction

This Framework Protocol has been prepared to facilitate the conduct of field trials and effective of biological evaluation tests on cereals (sorghum, millet and maize) stem borer insects in all Member States of ECOWAS, CILSS and UEMOA.

It describes the principles for the for the conduct of biological evaluation test on new active ingredients or insecticide formulations designed to control sorghum, millet and maize stem borer insects (Lepidoptera stem borers and shoot flies)

Approvals and Amendments

Initial Approval by WAPRC: ..... July 2008, Reference No. PC 2 PS 18_ECOWAS

1.0 Experimental Conditions

1.1 Target Pest, Selection of Crops and Varieties

The trial must be carried out in a well developed plantation of fruit trees (mango, or citrus trees) along with one or more fruit flies susceptible varieties. The trial must start at the beginning of flowering. Soil type must be uniform throughout the field.

1.2 Test Conditions

1.2.1 Test Conditions

The test should be carried out in areas where the fruit fly pest pressure is generally high. The trial must be carried out on fruit tree varieties sensitive to flies. Culture conditions (type of soil, fertilizer application, tillage, cultivar, row spacing) should be uniform in all the test plots and consistent with good agriculture practices. The cropping history and pesticide products applied in the preceding two years must be known and recorded.

The test should be conducted in similar or distinct agro-climatic conditions preferably in different years or growing seasons. It is important to conduct at least three independent tests in each agro-ecological zone (Sahel, Sudan and guinea etc).

For the purpose of obtaining provisional registration, the test should be conducted for two years on-site and for one year in on-farm as spelt out hereunder:

Year one: on-station test. Trial must be conclusive and reliable.

Year two: on-station and on-farm test at different agro ecological zones.

For the purpose of registration, two additional tests in on-farm conditions are necessary. It proposed that the tests be conducted by different institutions or individuals accredited by WAPRC.

1.3 Experimental Procedures and Conduct of Tests

1.3.1 On-station test

Treatments: The treatments consist of different rates (generally three) of the product under study, the rate of the manufacturer, ¾ and 3/2 of these rates, a reference product known for its efficacy against fruit flies and with similar mode of action to that of the product under study and an untreated control. The experimental design should be randomised complete block design. The plot size should generally be at least five trees, but evaluation of damage will be done only on the middle tree. At least one untreated control tree must separate various treatments. Three should be 4 replications.

1.3.2 On-farm test

Treatment: The treatments are generally made up of three rates. The best rate of the product derived from on-site tests, the rate proposed by the manufacturer and an untreated control. The plot size should generally be $100m^2$. Provision should be made for 5 replications.

2.0 Application of Treatment

2.1 Product(s) Under Study

The product to be tested should be a formulated product and should have a specific name.

2.2 Reference Product

In view of the variability of conditions under which pesticides are used, it is necessary to include a reference product in field trials to allow meaningful evaluation of efficacy under the conditions of the trial. Satisfactory levels of efficacy will generally be met when performance is comparable to that of the reference product, which should preferably be a registered product widely accepted as satisfactory in practice. However, other considerations (e.g. manner of use, side effects, etc may arise in assessing what is a satisfactory level of efficacy.

Where the type of pesticide product or its use is new, comparison with a reference product may be impossible or inappropriate. In this case, the product under study should show a consistent well-defined benefit. The pesticide to be introduced should be able to bring and/or keep the pest population and the damage to which it gives rise below an economic or phytosanitary threshold level, where this is known.

Wherever feasible the reference product chosen should be one which has shown satisfactory results in practice and its mode of action should be the same as or similar to that of the test product. The registration authority must be consulted on issues relating to the selection of a reference product for the study. Where a there is the need to use a reference product other than the one agreed or recommended by the registration authority, a justification must be made.

2.3 Untreated Control

An untreated control is needed for all trials both on-site tests and on-station or on-farm.

2.4 Application Methods

Application should conform to good agriculture practice.

2.4.1 Method of application

The recommended method of application should be used.

2.4.2 Type of application equipment

Each application should be done with the aid of equipment that ensures uniform distribution of the product over the entire plot or that ensures guided target treatment. Factors likely to affect efficiency of delivery such as pressure or nozzle type should be avoided. The equipment should be selected in accordance with product recommendation.

2.4.3 Timeframe and frequency of application

The number of applications and date of each treatment should be as those indicated for the proposed usage. These will depend on the biology of the target pest. The date of the applications should be recorded.

2.4.4 Application rate and volumes

The product should be tested using the recommended rate(R). With respect to efficacy tests, at least two (2) supplementary rates should be used, a lower and a higher rate. The exact choice of rate is expected to help determine whether the rate recommended by the manufacturer is the optimal rate in terms of efficacy and economic returns under a given agro-ecological conditions.

In on-farm setting, the optimal rate determined from on-site test is generally tested.

The rate should be expressed in kg/ha or l/ha of the formulated product and in g ai/h for the active ingredient. It may also be necessary to express this dose in g ai/kg or g.ai/l. For formulations in powder for dust spraying, be it granulated or similar products; this should be expressed in g ai/kg or %.

For seed treatment, the rate should be expressed in kg (or litres) of the formulated product per ton of seeds, and in g ai/kg of seeds.

The actual rate applied should always be measured and any deviation from the recommended rate recorded.

2.4.5 Information on all other pesticide products

Where other pesticide products (or biological control agents) are used, they should be applied uniformly on all plots. They should not be mixed with the product under study and the reference product. The date on which the treatments were applied should be recorded. The risk of interference should be minimized.

3.0 Observations and Data Collection, Recordings and Measurements

3.1 Meteorological Data

Days before and after product application, the meteorological data likely to influence crop development and/or that of the target pest and the action of the pesticide product should be recorded. Such data should normally include rainfall and temperature. All data should in principle, be recorded on the test site, especially for on-site test. It is sometimes difficult to obtain meteorological data in the local on-farm condition in which case, the relevant data could be obtained from the nearest meteorological station.

On the day of application, the meteorological data likely to influence the quality and persistency of the product should be recorded. Such data will normally include at least rainfall (nature and volume in mm) and temperature (mean maximum and minimum in ^{o}C) any significant change in the days weather should be recorded and in relation to the

application indicated.

Throughout the test period, prolong dry periods, heavy rains, sandy winds etc, likely to influence the outcome of the test should be recorded. Accurate data should be provided on any irrigation to be carried out on the plot.

3.1.1 Edaphic data

Not required.

3.2 Method, Time and Frequency of Observation

The growth stage of the crop should be recorded during each application.

3.2.1 Method of assessing fruit damage

At least 50-100 mature fruits (or all fruits from the tree) are harvested and examined for sign of oviposition. It is also possible to count the fruits that dropped (by distinguishing the fruits whose fall were caused by flies and those that fell for other reasons) furthermore; the count can be based on the trapping of adults. To do that, pheromone traps are positioned in each plot 24 hours after application to count the number of adults captured.

3.2.2 Timeframe and frequency of observation

Data collection and observation is done 3,7,10 and 14 days after treatment.

3.3 Observations of Phytotoxicity

Trees must be examined for the presence or absence of phytotoxicity on/from the leaves. Where toxicity effects are measurable, count the number of leaves showing the symptoms. The results must be indicated in absolute figures. Otherwise, the damages caused by phytotoxicity can be estimated based on a rating scale as against the untreated plots.

In any case the symptoms of burning must be well described: chlorosis, faltering, deformation.

3.4 Observations of Effects on Non-Target Organism

3.4.1 Effects on other pest

All observed effects on other pest whether positive or negative should be recorded.

3.4.2 Effects on beneficial organisms

Any positive or negative effect observed on parasitoids and predators, pollinators on adjacent or succeeding crops should be recorded. Any effect on the environment especially the effects on other fauna (both terrestrial and aquatic) should be recorded.

3.5 Quantitative and Qualitative Evaluation Of Yield

- Examine and determine the percentage marketable fruits out of 50-100 harvested fruits.
- Determine the weight of marketable fruits per plot.

4.0 Results

Test results should be presented in systematic and easily understandable form. They should be subjected to statistical analysis using pre-established methods. The report should include analysis and interpretation of the relevant data. Other stages of the evaluation will then follow. *Vide* OEPP PP 1/152 (2) standard - . Directive for the conduct and analysis of

biological evaluation tests and OEPP PP 1/181 standard - Directive on biological evaluation of phytosanitary products for the conduct of biological evaluation tests and presentation of reports.

CHAPTER 20

PROTOCOL FOR BIOLOGICAL EVALUATION OF PESTICIDES TO CONTROL COWPEA (NIEBE) LEAF FEEDING INSECTS

Introduction

This specific protocol has been prepared to facilitate the conduct of field trials and effective comparison of test results in all Member States of ECOWAS, CILSS and UEMOA.

This section defines the general principles for biological evaluation of new active ingredients or formulations of insecticides designed to control insects that feed on cowpea (niebe) leaves.

Approvals and Amendments

Initial Approval by WAPRC: ..... July 2008 Reference No. PC 2 PS 13_ECOWAS.

1.0 Experimental Conditions

1.1 Target Pest, Selection of Crops and Varieties

The relevant insects are: Jassids(*Empoasca sp*), (*Aphis craccivora*), *aleruca(Ootheca mutabilis; Medythia quaterna)*
The crop under cultivation is cowpea (*Vigna unguiculata*)
It is important to take into consideration the biology and ecology of each species used in the conduct of the experiment. Thus the test should be conducted when there is high pest pressure of the pest concerned or the borer species, to ensure that the high infestation period coincide with the stages.
The test should take into account the different species and their stages of development.

1.2 Test Conditions

The test should be carried out in areas where the pest pressure is generally high. The cropping condition (type of soil, fertilizer application, tillage, cultivar, row spacing) should be uniform in all the test plots and consistent with good agriculture practices. The cropping history and pesticide products applied in the preceding two years must be known and recorded.

The test should be conducted in similar or distinct agro-climatic conditions preferably in different years or growing seasons. It is important to conduct at least three independent tests in each agro-ecological zone (Sahel, Sudan and guinea etc). For the purpose of obtaining provisional registration, the test should be conducted for two years on-site and for one year in on-farm as spelt out hereunder:

Year one: on-site test. This should be conclusive with reliable results.

Year two: on-site test/on-farm, taking the agro ecological zoning into account.

For the purpose of registration, two additional tests in on-farm conditions are necessary. It proposed that the tests be conducted by different institutions or individuals accredited by WAPRC.

1.3 Experimental Procedures and Conduct of Tests

1.3.1 On-site test

Treatments: The treatments consist of different rates (generally three) of the product under study, a reference product and an untreated control. The experimental design should be randomised complete block design. The plot size should generally be $25m^2$ with 3m spacing between the plots. At the least 4 replications are required for each treatment.

1.3.2 on-farm test

Treatment: The treatments are generally made up of three rates. The best rate of the product derived from on-site tests, the rate proposed by the manufacturer and an untreated control. The plot size should generally be $100m^2$. Owing to the wide variations between the plots and the possibility of losing some of the treatments, at least 5 replications are required for every test. Enough space should be provided (3m) between the plots taking into account the wind direction during treatments.

2.0 Application of Treatment

2.1 Product(s) Under Study

The product to be tested should be a formulated product and should have a specific name.

2.2 Reference Product

In view of the variability of conditions under which pesticides are used, it is necessary to include a reference product in field trials to allow meaningful evaluation of efficacy under the conditions of the trial. Satisfactory levels of efficacy will generally be met when performance is comparable to that of the reference product, which should preferably be a registered product widely accepted as satisfactory in practice. However, other considerations (e.g. manner of use, side effects, etc may arise in assessing what is a satisfactory level of efficacy.

Where the type of pesticide product or its use is new, comparison with a reference product may be impossible or inappropriate. In this case, the product under study should show a consistent well-defined benefit. The pesticide to be introduced should be able to bring and/or keep the pest population and the damage to which it gives rise below an economic or phytosanitary threshold level, where this is known.

Wherever feasible the reference product chosen should be one which has shown satisfactory results in practice and its mode of action should be the same as or similar to that of the test product. The registration authority must be consulted on issues relating to the selection of a reference product for the study. Where a there is the need to use a reference product other than the one agreed or recommended by the registration authority, a justification must be made.

2.3 Untreated Control

An untreated control is needed for on-site tests and also for on-farm. Test should be conducted under local farming conditions.

2.4 Application Methods

Application should conform to good agriculture practice.

2.4.1 Method of application

The recommended method of application should be used.

2.4.2 Type of application equipment

Each application should be done with the aid of equipment that ensures uniform distribution of the product over the entire plot or that ensures guided target treatment. Factors likely to modify efficiency of delivery such as pressure or nozzle type should be avoided. The equipment should be selected in accordance with product recommendation.

For stem cuttings, treatment should be carried out with equipment which ensures uniform distribution of the product in accordance with recommended practice.

2.4.3 Timeframe and frequency of application

The number of applications and date of each treatment should be as those indicated for the proposed usage. These will depend on the stage of development of the crop and the biology of the target pest (stage of development, tolerance threshold and population peak. The date of the applications should be recorded.

2.4.4 Application rates and volumes

On-site: a minimum of three rates should be tested, the manufacturers rate, a lower rate and a higher rate. The exact choice of rate is expected to help determine if the rate proposed by the manufacturer is the optimal rate in terms of effectiveness and economic returns under humid/Sahel conditions.
Local farming setting: the optimal rate ascertained from the on-site test is generally tested.
The applicable rate should be expressed in g ai/ha and in kg/ha or l/h for the formulated product. For liquid formulations, the concentration should be in g ai/l.

For formulations in powder for dust spraying, be it granulated or similar products; this should be expressed in g ai/kg or %.

The actual rate applied should always be measured and any deviation from the recommended rate recorded.

2.4.5 Information on other pesticide products

Where other pesticide products (or biological control agents) are used, they should be applied uniformly on all plots. They should not be mixed with the product under study and the reference product. The date on which the treatments were applied should be recorded. The risk of interference should be minimized.

3.0 Observations and Data Collection, Recordings and Measurements

3.1 Meteorological and Edaphic Data

Days before and after product application, the meteorological data likely to influence crop development and/or that of the target pest and the action of the pesticide product should be recorded. Such data should normally include rainfall and temperature. All data should in principle, be recorded on the test site, especially for on-site test. It sometimes difficult to obtain meteorological data in the local on-farm condition in which case, the relevant data could be obtained from the nearest meteorological station.

On the day of application, the meteorological data likely to influence the quality and persistency of the product should be recorded. Such data will normally include at least rainfall (nature and volume in mm) and temperature (mean maximum and minimum in ^{o}C)

any significant change in the days weather should be recorded and in relation to the application indicated.

Throughout the test period, prolong dry periods, heavy rains, sandy winds etc, likely to influence the outcome of the test should be recorded. Accurate data should be provided on any irrigation to be carried out on the plot.

3.1.1 Edaphic data

These are not required.

3.2 Method, Observation and Data Collection

The growth stage of the crop should be recorded during each application and observation.

3.2.1 Method

The sampling method or observation method should depend on the pest under consideration. The method chosen should lend itself to statistical evaluation to assess the efficacy of the product. Sampling should be made three times a week on 4 middle rows of the plots. The samples should comprise 200 leaves (50 leaves per row x 4) middle rows tagged plants marked with red colour thread at embryo stage. The date of 50%emergence and 50% flowering should be recorded. From the emergence to flowering, the number of insects on tagged plants should be counted two times a week. The sampling period should be between 6.00am and 7.00am and between 5.00pm and 6.00pm.
The population of the insects that damage the plants should be taken into account and the level of infestation assessed by counting the number of perforated leaves with the damaged leaves classified according to the rating scale 0-7. Where 0=5%, 1=15%, 2=25%, 3=35%, 4=45%, 5=60%, 6=85%, 7=100%.

3.2.2. Timeframe and frequency of observation

At least one observation in required on pest infestation and damage (generally 1-3 days) before the first treatment during the growing season. A sampling should be carried out to determine species composition and degree of infestation. 2-7days after treatment, a sample should be taken to assess the level of population in relation to the initial pre-treatment. The timeframes and frequencies of observation should take into account the growth phonological stage of the crop and the level of pest population, stage of development of the pest the rate of product action and persistence.

3.3 Observations of Phytotoxicity

Phytotoxicity should be examined and all positive or negative effects recorded.

3.4 Observations of eEfects on Non-target Organism

3.4.1 Effects on other pest

All observed effects on other pest are it positive or negative should be recorded.

3.4.2 Effects on beneficial organisms

Any positive or negative effect observed on parasitoids and predators, pollinators on adjacent or succeeding crops should be recorded. Any effect on the environment especially the effects on other fauna (both terrestrial and aquatic) should be described.

3.5 Quantitative and Qualitative Evaluation of Harvested Crop

Although it is recognized that crop yield depends on several factors, quantitative and/or qualitative assessment of harvested crop is required.

4.0 Results

Test results should be presented in systematic and easily understandable form. They should be subjected to statistical analysis using pre-established methods. The report will include analysis and interpretation of the relevant data. Other stages of the evaluation will then follow. *Vide* OEPP PP 1/152 (2) standard Directive for the conduct and analysis of biological evaluation tests and OEPP PP 1/181 standard - Directive on biological evaluation of phytosanitary products for the conduct of biological evaluation tests and presentation of reports.

CHAPTER 21

SPECIFIC PROTOCOL FOR BIOLOGICAL EVALUATION OF PESTICIDES TO CONTROL COTTON SUCKING INSECTS

Introduction

This Specific Protocol has been developed to facilitate the conduct of experiments and comparison of the results of biological evaluation test on new active ingredients or formulations of the pesticides to control cotton sucking insects in all Member States of ECOWAS, CILSS and UEMOA.

Approvals and Amendments

Initial approval by *WAPRC*; …July, 2008, Reference No. PC 2 PS 03_ ECOWAS

1.0 Experimental Conditions

1.1 Target Pest, Selection of Crops and Varieties

The important cotton sucking insects are as follows:

- *Aphis gossypii* (GLOVER)
- *Bemisia Tabaci* (GENNADIUS)
- *Jacobiasca lybica* (BERGEVIN & ZANON) and
- *Jacobiella fascialis* (JACOBI)

The above list may also include Bryobia, the most dangerous of which is Bulb scale mite- *Polyphagotarsonemus latus* (BANKS). The following insect could also be taken into consideration, exceptionally, where they are of significance. These are mirids or bugs (*Campylomma spp, Megacoelum spp,* etc.) Pentatomides (*Aspavia sp, Piezodorus sp*) and *Pyrrocorids (Dysdercus sp)*

The biology of the species should be taken into consideration in the timing of the experiment. This mean in particular, conducting the test at the periods when crops are likely to be subject to significant pest infestation. The tests should be carried out on the organisms and stages of development of the organism so tested should be indicated in the proposed usage.

The relevant plant is cotton plant (*Gossypium hirsutum)*. Two types of cotton are cultivated in the sub-region. The cotton plant with gossypol glands is by far the most widespread. The cotton plant without gossypol glands is also cultivated but at lesser scale. The species are numerous and at times vary from country to country.

1.2 Test Conditions

1.2.1 Test Conditions

The test should be carried out in areas where the pest pressure is generally high. The cropping condition (type of soil, fertilizer application, tillage, cultivar, row spacing) should be uniform

in all the test plots and consistent with good agriculture practices. The cropping history and pesticide products applied in the preceding two years must be known and recorded.

The test should be conducted in similar or distinct agro-climatic conditions preferably in different years or growing seasons. It is important to conduct at least three independent tests in each agro-ecological zone (Sahel, Sudan and guinea etc). For the purpose of obtaining provisional registration, the test should be conducted for two years on-site and for one year in on-farm as spelt out hereunder:

Year one: on-site test this should be conclusive with reliable results.

Year two: on-site test and on-farm, taking the agro ecological zoning into account.

For the purpose of registration, two additional tests in on-farm conditions are necessary. It proposed that the tests be conducted by different institutions or individuals accredited by WAPRC.

1.3 Experimental Procedures and Conduct of Tests

1.3.1 On-site test

Treatments: The treatments consist of different rates (generally three) of the product under study, a reference product and an untreated control. The experimental design should be randomised complete block design. The plot size should generally be 8 lines of 15m long with 0.8m row width. These plots are separated by 15m long 3 line buffer area. The number of replications varies from 4 to 8.

1.3.2 On-farm test:

Treatments: The treatments consist of different rates (generally three) of the product under study, a reference product and an untreated control. The experimental design should be randomised complete block design. The plot size should generally be ¼ to ½ ha. The buffer area is optional. The numbers of replications vary from 6 to 10.

2.0 Application of Treatment

2.1 Product(s) Under Study

The product to be tested should be a formulated product and should have a specific name.

2.2 Reference Product

In view of the variability of conditions under which pesticides are used, it is necessary to include a reference product in field trials to allow meaningful evaluation of efficacy under the conditions of the trial. Satisfactory levels of efficacy will generally be met when performance is comparable to that of the reference product, which should preferably be a registered product widely accepted as satisfactory in practice. However, other considerations (e.g. manner of use, side effects, etc may arise in assessing what is a satisfactory level of efficacy.

Where the type of pesticide product or its use is new, comparison with a reference product may be impossible or inappropriate. In this case, the product under study should show a consistent well-defined benefit. The pesticide to be introduced should be able to bring and/or keep the pest population and the damage to which it gives rise below an economic or phytosanitary threshold level, where this is known.

Wherever feasible the reference product chosen should be one which has shown satisfactory results in practice and its mode of action should be the same as or similar to that of the test product. The registration authority must be consulted on issues relating to the selection of a reference product for the study. Where a there is the need to use a reference product other than the one agreed or recommended by the registration authority, a justification must be made.

2.3 Untreated Control

An untreated control is needed for on-site tests and also for on-farm conditions.

2.4 Application Methods

Application should conform to good agriculture practice.

2.4.1 Method of application

The method of application should be chosen depending on the magnitude of the test. For test on small areas, atomized sprayers (preferably hand operated) with 80-120 litres/ha of spray material should be used.

For large-scale test, very low volume appliances could be used. The volumes of spray material administered should be equivalent to 10l/ha at least.

2.4.2 Type of application equipment

Each application should be done with the aid of equipment that ensures uniform distribution of the product over the entire plot or that ensures guided target treatment. Factors likely to modify efficiency of delivery such as pressure or nozzle type should be avoided. The equipment should be selected in accordance with product recommendation.

2.4.3 Timeframe and frequency of application

The number of applications and the date of each treatment should be indicated. In very many cases, the treatment should depend on how high or low the infestation rate. In the case of plant aphid, treatment should be carried out between the flower emergence and floral bud stage, when 8 – 10 % of the plants are infested.

2.4.4 Application rates and volumes

On-site: a minimum of three rates should be tested, the manufacturers rate, a lower rate and a higher rate. The exact choice of rate is expected to help determine if the rate proposed by the manufacturer is the optimal rate in terms of effectiveness and economic returns under humid conditions.
Local farming setting: the optimal rate ascertained from the on-site test is generally tested.
The applicable rate should be expressed in kg or l of the formulated product per ha. As well as g ai per ha. For liquid formulations in powder for dust spraying, be it granular or similar products, the data should be presented in g ai/kg or %.
The actual rate applied should always be measured and any deviation from the recommended rate recorded.

2.4.5 Information on other pesticide products

Where other pesticide products (or biological control agents) are used, they should be applied uniformly on all plots. They should not be mixed with the product under study and the reference product. The date on which the treatments were applied should be recorded.

The risk of interference should be minimized.

3.0 Observations and Data Collection, Recordings and Measurements

3.1 Meteorological and Edaphic Data

Days before and after product application, the meteorological data likely to influence crop development and/or that of the target pest and the action of the pesticide product should be recorded. Such data should normally include rainfall and temperature. All data should in principle, be recorded on the test site, especially for on-site test. It sometimes difficult to obtain meteorological data in the local on-farm condition in which case, the relevant data could be obtained from the nearest meteorological station.

On the day of application, the meteorological data likely to influence the quality and persistency of the product should be recorded. Such data will normally include at least rainfall (nature and volume in mm) and temperature (mean maximum and minimum in ^{o}C) any significant change in the days weather should be recorded and in relation to the application indicated.

Throughout the test period, prolong dry periods, heavy rains, sandy winds etc, likely to influence the outcome of the test should be recorded. Accurate data should be provided on any irrigation to be carried out on the plot.

3.1.1 Edaphic data

These are not required.

3.2 Method, Observation and Data Collection

The growth stage of the crop should be recorded during each application and observation.

3.2.1 Method

The number of sucking insects should be counted or estimated on at least five leaves per plant and for between 15 and 20 plants, in the middle row of each test plot.

For aphids, where over a hundred insects are present per leaf (per half-leaf) with high insect population, visual estimate can be undertaken in accordance with pre-determined rating scale.

For white flies, the larvae, pupae and adult should be counted. Here also, visual observation could be done in case of high insect population.

3.2.2 Timeframe and Frequency

- Preliminary observation: just before application
- First observation: 3 days after application
- Second observation : 7 days after application
- Third observation:14 days after application

If need be, the observations could continue at weekly intervals.

3.3 Observation of Phytotoxicity on the Crop

Phytotoxicity should be examined and all positive or negative effects recorded. Phytotoxicity effects should be assessed in the following manner:

1. Where the effects can be counted or measured, the results should be indicated in absolute figures.
2. In other cases, the frequency and intensity of damage should be assessed in any of the following ways:

- Phytotoxicity in each plot is assessed by reference to a rating scale: or
- Each treated plot is compared to an untreated control plot and the percentage of toxicity estimated.

In all cases, the phytotoxicity symptoms (stunting, chlorosis or yellowing, deformation etc) should be clearly recorded.

3.4 Observations of Effects on Non-Target Organism

3.4.1 Effects on other pest

All observed effects on other pest are it positive or negative should be recorded.

3.4.2 Effects on beneficial organisms

Any positive or negative effect observed on parasitoids and predators, pollinators on adjacent or succeeding crops should be recorded. Any effect on the environment especially the effects on other fauna (both terrestrial and aquatic) should be described.

3.5 Quantitative and Qualitative Evaluation of Harvested crop

This is required only where the differences are significant. In that case, the estimated yield should be noted in kg/ha of seed cotton..

- Number of bolls/plant
- Number of open bolls/plant

4.0 Results

Test results should be presented in methodic and easily understandable form. They should be submitted for statistical analysis using pre-established methods. The report will include analysis and interpretation of the data. Other stages of evaluation will then follow. Vide OEPP PP 1.152 (2) standard - Directive for the conduct and analysis of biological evaluation tests and OEPP PP 1/181 standard- Directive on biological evaluation of phytosanitary products for the conduct of biological evaluation tests and presentation of reports.

CHAPTER 22

PROTOCOL FOR BIOLOGICAL EVALUATION OF PESTICIDES TO CONTROL COTTON LEAF FEEDING PESTS

Introduction

This Framework Protocol has been prepared to facilitate the conduct of field trials and effective comparison of test results in all Member States of ECOWAS, CILSS and UEMOA.

It describes the principles for the conduct of biological evaluation test on new active ingredients or insecticide formulations designed to control Cotton leaf eating pest.

Approvals and Amendments

Initial Approval by WAPRC: ..... July 2008, Reference No. PC2 PS 02_ECOWAS

1.0 Experimental Conditions

1.1 Target Pest, Selection of Crops and Varieties

The important cotton leaf feeding insects are:

- *Cosmophila (=Anomis) flava(F)*
- *Syllepte (sylepta derogate)(F)*
- *Spodoptera littoralis(*BOISDUVAL) which also attacks fruiting organs of cotton plant.
- *Xanthodes graelllsi(*FEISTHAMEL)
- *Plusia(=Chrysodexis) spp.*
- *Spodoptera exigua(*LAPHEG)
- *Amsacta sp*
- *Diacrisia sp.*

This list includes:
Orthorptera represented by *Oecanthus sp* and *Zonocerus variegates (*L), Coleoptera including Chrysomelids eg Nisotra dilecta (DALMAN) and *Podogrica uniformis* (JACOB)

The biology of the species should be taken into consideration in the timing of the experiment. This mean in particular, conducting the test at the periods when crops are likely to be subject to significant pest infestation. The tests should be carried out on the organisms and stages of development of the organism so tested should be indicated in the proposed usage.

The relevant plant is cotton plant (*Gossypium hirsutum)*. Two types of cotton are cultivated in the sub-region. The cotton plant with gossypol glands is by far the most widespread. The cotton plant without gossypol glands is also cultivated but at lesser scale. The species are numerous and at times vary from country to country.

1.2 Test Conditions

1.2.1 Test Conditions

The test should be carried out in areas where the pest pressure is generally high. The cropping condition (type of soil, fertilizer application, tillage, cultivar, row spacing) should be uniform in all the test plots and consistent with good agriculture practices. The cropping history and pesticide products applied in the preceding two years must be known and recorded.

The test should be conducted in similar or distinct agro-climatic conditions preferably in different years or growing seasons. It is important to conduct at least three independent tests in each agro-ecological zone (Sahel, Sudan and guinea etc). For the purpose of obtaining provisional registration, the test should be conducted for two years on-site and for one year in on-farm as spelt out hereunder:

Year one: on-site test this should be conclusive with reliable results.

Year two: on-site test and on-farm, taking the agro ecological zoning into account.

For the purpose of registration, two additional tests in on-farm conditions are necessary. It proposed that the tests be conducted by different institutions or individuals accredited by WAPRC.

1.3 Experimental Procedures and Conduct of Tests

1.3.1 On-site test

Treatments: The treatments consist of different rates (generally three) of the product under study, a reference product and an untreated control. The experimental design should be randomised complete block design. The plot size should generally be 8 lines of 15m long with 0.8m row width. These plots are separated by 15m long 3 line buffer area. The number of replications varies from 4 to 8.

1.3.2 On-farm test:

Treatments: The treatments consist of different rates (generally three) of the product under study, a reference product and an untreated control. The experimental design should be randomised complete block design. The plot size should generally be ¼ to ½ ha. The buffer area is optional. The numbers of replications vary from 6 to 10.

2.0 Application of Treatment

2.1 Product(s) Under Study

The product to be tested should be a formulated product and should have a specific name.

2.2 Reference product

In view of the variability of conditions under which pesticides are used, it is necessary to include a reference product in field trials to allow meaningful evaluation of efficacy under the conditions of the trial. Satisfactory levels of efficacy will generally be met when performance is comparable to that of the reference product, which should preferably be a registered product widely accepted as satisfactory in practice. However, other considerations (e.g. manner of use, side effects, etc may arise in assessing what is a satisfactory level of efficacy.

Where the type of pesticide product or its use is new, comparison with a reference product may be impossible or inappropriate. In this case, the product under study should show a consistent well-defined benefit. The pesticide to be introduced should be able to bring and/or keep the pest population and the damage to which it gives rise below an economic or phytosanitary threshold level, where this is known.

Wherever feasible the reference product chosen should be one which has shown satisfactory results in practice and its mode of action should be the same as or similar to that of the test product. The registration authority must be consulted on issues relating to the selection of a reference product for the study. Where a there is the need to use a reference product other than the one agreed or recommended by the registration authority, a justification must be made.

2.3 Untreated Control

An untreated control is needed for on-site tests and also for on-farm conditions.

2.4 Application Methods

Application should conform to good agriculture practice.

2.4.1 Method of application

The method of application should be chosen depending on the magnitude of the test. For test on small areas, atomized sprayers (preferably hand operated) with 80-120 litres/ha of spray material should be used.

For large scale test, very low volume appliances could be used. The volumes of spray material administered should be equivalent to 10l/ha at least.

2.4.2 Type of application equipment

Each application should be done with the aid of equipment that ensures uniform distribution of the product over the entire plot or that ensures guided target treatment. Factors likely to modify efficiency of delivery such as pressure or nozzle type should be avoided. The equipment should be selected in accordance with product recommendation.

2.4.3 Timeframe and frequency of application

The number of applications and the date of each treatment should be indicated. In very many cases, the treatment should in principle be done when the concentration of the target pest population is high. For example treatment should be carried out after massive hatching of the eggs at an average density of at least 1 larva per plant (or 5% of the leaves infested)

2.4.4 Application rates and volumes

On-site: a minimum of three rates should be tested, the manufacturers rate, a lower rate and a higher rate. The exact choice of rate is expected to help determine if the rate proposed by the manufacturer is the optimal rate in terms of effectiveness and economic returns under humid conditions.

Local farming setting: the optimal rate ascertained from the on-site test is generally tested.
The applicable rate should be expressed in kg or l of the formulated product per ha. As well as g ai per ha. For liquid formulations in powder for dust spraying, be it granular or similar products, the data should be presented in g ai/kg or %.
The actual rate applied should always be measured and any deviation from the

recommended rate recorded.

2.4.5 Information on other pesticide products

Where other pesticide products (or biological control agents) are used, they should be applied uniformly on all plots. They should not be mixed with the product under study and the reference product. The date on which the treatments were applied should be recorded. The risk of interference should be minimized.

3.0 Observations and Data Collection, Recordings and Measurements

3.1 Meteorological and Edaphic Data

Days before and after product application, the meteorological data likely to influence crop development and/or that of the target pest and the action of the pesticide product should be recorded. Such data should normally include rainfall and temperature. All data should in principle, be recorded on the test site, especially for on-site test. It sometimes difficult to obtain meteorological data in the local on-farm condition in which case, the relevant data could be obtained from the nearest meteorological station.

On the day of application, the meteorological data likely to influence the quality and persistency of the product should be recorded. Such data will normally include at least rainfall (nature and volume in mm) and temperature (mean maximum and minimum in ^{o}C) any significant change in the days weather should be recorded and in relation to the application indicated.

Throughout the test period, prolong dry periods, heavy rains, sandy winds etc, likely to influence the outcome of the test should be recorded. Accurate data should be provided on any irrigation to be carried out on the plot.

3.1.1 Edaphic data

These are not required.

3.2 Method, Observation and Data Collection

The growth stage of the crop should be recorded during each application and observation.

3.2.1 Method

Pest count

Count the number of larvae (distinguish their age if possible) and/or the leaves infested on 15 to 20 plants in the middle row of each plot. In case of damage, the following visual observations may be made. To this end, a rating scale below may be used.
0 = no damage
1 = up to 10% damage
2 = 10 to 25% damage
3 = 25 to 50% damage
4 = 0ver 50% damage

3.2.2. Timeframe and frequency of observation

- Preliminary observation: just before application.
- First observation: 3 days after application.

- Second observation: 7 days after application.
- Third observation: 14 days after application.

The observations could if need be continue at weekly intervals.

3.3 Observation of Phytotoxicity on the Crop

Phytotoxicity should be examined and all positive or negative effects recorded. Phytotoxicity effects should be assessed in the following manner:

3. Where the effects can be counted or measured, the results should be indicated in absolute figures.
4. In other cases, the frequency and intensity of damage should be assessed in any of the following ways:

- Phytotoxicity in each plot is assessed by reference to a rating scale: or
- Each treated plot is compared to an untreated control plot and the percentage of toxicity estimated.

In all cases, the phytotoxicity symptoms (stunting, chlorosis or yellowing, deformation etc) should be clearly recorded.

3.4 Observations of Effects On Non-Target Organism

3.4.1 Effects on other pest

All observed effects on other pest are it positive or negative should be recorded.

3.4.2 Effects on beneficial organisms

Any positive or negative effect observed on parasitoids and predators, pollinators on adjacent or succeeding crops should be recorded. Any effect on the environment especially the effects on other fauna (both terrestrial and aquatic) should be described.

3.5 Quantitative and Qualitative Evaluation of Harvested Crop

This is required only where the differences are significant. In that case, the estimated yield should be noted in kg/h of cotton seed.

4.0 Results

Test results should be presented in systematic and easily understandable form. They should be subjected to statistical analysis using pre-established methods. The report should include analysis and interpretation of the relevant data. Other stages of evaluation will then follow. Vide OEPP PP1/152(2) standard-directive for the conduct and analysis of biological evaluation tests and OEPP PP 1/181 standard-Directive on biological evaluation of pesticide product for the conduct of biological evaluation tests and presentation of reports.

CHAPTER 23

PROTOCOL FOR BIOLOGICAL EVALUATION OF PESTICIDES TO CONTROL COTTON BOLLWORMS

Introduction

This Framework Protocol has been prepared to facilitate the conduct of field trials and effective comparison of test results in all Member States of ECOWAS, CILSS and UEMOA.

It describes the principles for the conduct of biological evaluation test on new active ingredients or insecticide formulations designed to control Cotton Bollworms.

Approvals and Amendments

Initial Approval by WAPRC: ..... July 2008, Reference No. PC2 PS 01_ECOWAS

1.0 Experimental Conditions

1.1 Target Pest, Selection of Crops and Varieties

The important cotton bollworms are:

- *Helicoverpa armigera(*HUBNER*)*
- *Diparopsis watersi(*ROTHSCHILD)
- *Earias insulana(*BOISDUVAL) *et Earias biplaga(*WALKER)
- *Spodoptera leucotreta(*BOISDUVAL)
- *Cryptophlebia leucotreta(*MERICK)
- *Pectinophora gossypiella(*SAUNDERS)

The biology of the species should be taken into consideration in the timing of the experiment. This mean in particular, conducting the test at the periods when crops are likely to be subject to significant pest infestation. The tests should be carried out on the organisms and stages of development of the organism so tested should be indicated in the proposed usage.

The relevant plant is cotton plant (*Gossypium hirsutum)*. Two types of cotton are cultivated in the sub-region. The cotton plant with gossypol glands is by far the most widespread. The cotton plant without gossypol glands is also cultivated but at lesser scale. The species are numerous and at times vary from country to country.

1.2 Test Conditions

1.2.1 Test Conditions

The test should be carried out in areas where the pest pressure is generally high. The cropping condition (type of soil, fertilizer application, tillage, cultivar, row spacing) should be uniform in all the test plots and consistent with good agriculture practices. The cropping history and pesticide products applied in the preceding two years must be known and recorded.

The test should be conducted in similar or distinct agro-climatic conditions preferably in different years or growing seasons. It is important to conduct at least three independent tests

in each agro-ecological zone (Sahel, Sudan and guinea etc). For the purpose of obtaining provisional registration, the test should be conducted for two years on-site and for one year in on-farm as spelt out hereunder:

Year one: on-site test this should be conclusive with reliable results.

Year two: on-site test and on-farm, taking the agro ecological zoning into account.

For the purpose of registration, two additional tests in on-farm conditions are necessary. It proposed that the tests be conducted by different institutions or individuals accredited by WAPRC.

1.3 Experimental Procedures and Conduct of Tests

1.3.1 On-site test

Treatments: The treatments consist of different rates (generally three) of the product under study, a reference product and an untreated control. The experimental design should be randomised complete block design. The plot size should generally be 8 lines of 15m long with 0.8m row width. These parcels are separated by 15m long 3 line buffer area. The number of replications varies from 4 to 8.

2. 0 Application of Treatment

2.1 Product(s) Under Study

The product to be tested should be a formulated product and should have a specific name.

2.2 Reference Product

In view of the variability of conditions under which pesticides are used, it is necessary to include a reference product in field trials to allow meaningful evaluation of efficacy under the conditions of the trial. Satisfactory levels of efficacy will generally be met when performance is comparable to that of the reference product, which should preferably be a registered product widely accepted as satisfactory in practice. However, other considerations (e.g. manner of use, side effects, etc may arise in assessing what is a satisfactory level of efficacy.

Where the type of pesticide product or its use is new, comparison with a reference product may be impossible or inappropriate. In this case, the product under study should show a consistent well-defined benefit. The pesticide to be introduced should be able to bring and/or keep the pest population and the damage to which it gives rise below an economic or phytosanitary threshold level, where this is known.

Wherever feasible the reference product chosen should be one which has shown satisfactory results in practice and its mode of action should be the same as or similar to that of the test product. The registration authority must be consulted on issues relating to the selection of a reference product for the study. Where a there is the need to use a reference product other than the one agreed or recommended by the registration authority, a justification must be made.

2.3 Untreated Control

An untreated control is needed for on-site tests and also for on-farm conditions.

2.4 Application Methods

Application should conform to good agriculture practice.

2.4.1 Method of application

The method of application should be chosen depending on the magnitude of the test. For test on small areas, atomized sprayers (preferably hand operated) with 80-120 litres/ha of spray material should be used.

For large scale test, very low volume appliances could be used. The volumes of spray material administered should be equivalent to 10l/ha at least.

2.4.2 Type of application equipment

Each application should be done with the aid of equipment that ensures uniform distribution of the product over the entire plot or that ensures guided target treatment. Factors likely to modify efficiency of delivery such as pressure or nozzle type should be avoided. The equipment should be selected in accordance with product recommendation.

2.4.3 Timeframe and frequency of application

The number of applications and the date of each treatment should be indicated. In very many cases, the treatment should in principle be done when the concentration of the target pest population is high. For example treatment should be carried out after massive hatching of the eggs at an average density of at least 1 larva per plant (or 5% of the leaves infested)

2.4.4 Application rates and volumes

On-site: a minimum of three rates should be tested, the manufacturers rate, a lower rate and a higher rate. The exact choice of rate is expected to help determine if the rate proposed by the manufacturer is the optimal rate in terms of effectiveness and economic returns under humid/Sahel conditions.
Local farming setting: the optimal rate ascertained from the on-site test is generally tested.
The applicable rate should be expressed in kg or l of the formulated product per ha. As well as g ai per ha. For liquid formulations in powder for dust spraying, be it granular or similar products, the data should be presented in g ai/kg or %.

The actual rate applied should always be measured and any deviation from the recommended rate recorded.

2.4.5 Information on other pesticide products

Where other pesticide products (or biological control agents) are used, they should be applied uniformly on all plots. They should not be mixed with the product under study and the reference product. The date on which the treatments were applied should be recorded. The risk of interference should be minimized.

3.0 Observations and Data Collection, Recordings and Measurements

3.1 Meteorological and Edaphic Data

Days before and after product application, the meteorological data likely to influence crop development and/or that of the target pest and the action of the pesticide product should be recorded. Such data should normally include rainfall and temperature. All data should in principle, be recorded on the test site, especially for on-site test. It sometimes difficult to obtain meteorological data in the local on-farm condition in which case, the relevant data could be obtained from the nearest meteorological station.

On the day of application, the meteorological data likely to influence the quality and persistency of the product should be recorded. Such data will normally include at least rainfall (nature and volume in mm) and temperature (mean maximum and minimum in ^{o}C) any significant change in the days weather should be recorded and in relation to the application indicated.

Throughout the test period, prolong dry periods, heavy rains, sandy winds etc, likely to influence the outcome of the test should be recorded. Accurate data should be provided on any irrigation to be carried out on the plot.

3.1.1 Edaphic data

These are not required.

3.2 Method, Observation and Data Collection

The growth stage of the crop should be recorded during each application and observation.

3.2.1 Method

Pest count

Cotton bollworms: the number of live worms on squares (blossom buds) and/or bolls for 15-20 cotton plants per plot should be counted. The various species should be counted separately.

Eggs: the number of eggs on young leaves and terminal buds of 20 plants per plot should be counted separately for the various species.

Damage assessment

Damage assessment could be carried out using two distinct techniques.

- Either directly on 15-20 cotton plants by counting the number of sprouting organs (squares and bolls) infested by the bollworms.
- Or on all the squares and bolls that have dropped along the entire row space per plot, by collecting them sorting them and then separating them by type and state of health.

3.2.2. Timeframe and frequency of observation

The observations should be carried out as follows:

- Preliminary counting: just before application.
- First counting: 2 days after application, counting the living larvae.
- Second counting: 7 days after application, count the living larvae.
- Third counting: 14 days after application, count damaged bolls.

These procedures could be repeated at each application.

3.3 Observation of Phytotoxicity on the Crop

Phytotoxicity should be examined and all positive or negative effects recorded. Phytotoxicity effects should be assessed in the following manner:

5. Where the effects can be counted or measured, the results should be indicated in absolute figures.
6. In other cases, the frequency and intensity of damage should be assessed in any of the following ways:

- Phytotoxicity in each plot is assessed by reference to a rating scale: or
- Each treated plot is compared to an untreated control plot and the percentage of toxicity estimated.

In all cases, the phytotoxicity symptoms (stunting, chlorosis or yellowing, deformation etc) should be clearly recorded.

3.4 Observations of Effects on Non-Target Organism

3.4.1 Effects on other pest

All observed effects on other pest are it positive or negative should be recorded.

3.4.2 Effects on beneficial organisms

Any positive or negative effect observed on parasitoids and predators, pollinators on adjacent or succeeding crops should be recorded. Any effect on the environment especially the effects on other fauna (both terrestrial and aquatic) should be described.

3.5 Quantitative and Qualitative Evaluation of Harvested Crop

This is required only where the differences are significant. In that case, the estimated yield should be noted in kg/h of seed cotton.

Number of bolls/plant, number of open bolls/plant.

4.0 Results

Test results should be presented in systematic and easily understandable form. They should be subjected to statistical analysis using pre-established methods. The report should include analysis and interpretation of the relevant data. Other stages of evaluation will then follow. Vide OEPP PP1/152(2) standard-directive for the conduct and analysis of biological evaluation tests and OEPP PP 1/181 standard-Directive on biological evaluation of pesticide product for the conduct of biological evaluation tests and presentation of reports.

CHAPTER 24

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