MATERIALS
&
METHODS

II. MATERIALS AND METHODS

Survey for pathogens associated with white grub (H.consanguinea) :

Systematic surveys were conducted in white grub infested areas of Central and North Gujarat during 1985 to 1987. The grubs of H. consanguinea were periodically collected from fields of groundnut, sorghum, bajra, maize etc., and brought to the laboratory. They were reared individually in plastic container (3.5 x 7.5 cm) containing sterilized moist soil. Grubs were provided with washed roots of bajra as food. They were examined every day for disease development. The grubs, which became milky white were reared separately till they died. The dead grubs were examined for the presence of bacterium in the hemolymph. Grubs showing brown spots of

melanization were considered to be infected with fungus. After death, the hard cadavers were surface sterilized with 0.2% $HgCl_2$, placed in petridish (8.0 cm) containing moist filter paper and incubated for fungal growth. The milky grubs also showing brown spots were considered to be infected with fungal and bacterial pathogen together.

Identification of causative organisms :

During the course of survey the grubs were found to be infected by two pathogens, a fungus and a bacterium. The fungus was isolated as described by Mohan and Pillai (1982). Isolated fungus was further cultivated on Potato Dextrose Agar in the laboratory. It was identified as Metarhizium anisopliae (Metschn.) Sorokin var. anisopliae Dr. B.L. Brady, Commonwealth Mycological Institute, Kew, Surrey, England.

The hemolymph of milky disease grub was examined for the presence of bacteria under light microscope. The cadavers of milky diseased grubs were lyophilized in glass tubes with the help of lyophilizer. The lyophilized samples were sent to Dr. R.J. Milner of CSIRO, Canberra, Australia for identification of the causative bacteria. The organism was identified as Bacillus popilliae Dutky var. holotrichiae.

Of these pathogens, detailed investigations were made on B. popilliae var. holotrichiae.

Collection of white grubs :

To conduct laboratory experiments with the pathogen adults and grubs were collected. The adults of H. serrata were collected during March to June and while those of H. consanguinea, A. nathani and H. fregei were collected after the first monsoon rain in June to August from Anand and Kapadwanj areas which are endemic areas of the pest in Gujarat state. The adults were collected during 7.45 to 9.45 pm from the host trees viz., neam (Azadirachta indica), ber (Ziziphus jujuba) and drumstick (Moringa oleifera) with the help of insect nets using flash light. The grubs were collected by digging random pits (1 sq ft x 15 cm) in groundnut and cereal crop fields which were heavily infested with white grubs. These adults and grubs were brought to the laboratory and reared on neem leaves and bajra roots respectively for further experimental studies.

Rearing of white grub :

The adult beetles brought to laboratory were released in wooden cages (1 sq m in size and 60 cm in height) filled up to the depth of 30 cm with moist sandy loam soil. The cages were covered by black cotton cloth to avoid the escape of beetles. They were fed daily with fresh neem

leaves. The eggs laid by the beetles in the soil of cage were collected daily. The eggs were kept individually in plastic container measuring (3.5 cm x 7.5 cm) with some moist soil and examined daily to check for hatching of the eggs. After hatching of the eggs, bajra roots were provided as food for the grubs. To maintain the supply of sufficient fresh root material for the grubs in the laboratory, a thick mat of bajra seedlings was raised in shallow iron sheet trays (46.0 x 26.5 x 8.0 cm) containing some soil. The seedlings of about 5-8 cm in height were taken out along with the roots from a part of mat and planted in the soil of the bowl containing the grubs. The roots and seedlings remained fresh for about 24 hr and served as food for the grubs. Such laboratory reared grubs were used in the experiment.

Pathogenicity of B. popilliae var. holotrichiae against different species of white grub :

Laboratory experiments were conducted to test the pathogenicity of B. popilliae to different species of white grubs viz., H. consanguinea, H. serrata, H. fregei and A. nathani occurring in Gujarat. These grubs were reared individually in sterilized plastic containers containing moist soil contaminated with B. popilliae (1 x 10^7 spores/g of soil). The spores were obtained by aseptically bleeding field collected milky diseased grubs. Thoroughly washed

bajra roots were provided as food. The grubs thus reared were observed daily for a period of one month for development of the disease. The presence of B. popilliae in hemolymph of diseased grub was examined microscopically.

Mass propagation of B. popilliae var. holotrichiae :

Since the bacterium is an obligate pathogen, attempts were made to propagate the organism on the grubs of H. consanguinea. For this purpose, the grubs collected from the field or produced in the laboratory were inoculated by feeding on B. popilliae charged bajra roots and then reared individually in plastic containers until they died. Since production of diseased grubs was not found satisfactory, further attempts were made to improve the production of bacterium in the laboratory. Various methods for inducing milky disease caused by bacterium were tried and their effectiveness was evaluated.

For this purpose, the eggs laid by the H. consanguinea females collected during June - July from the fields were obtained by placing them in laboratory cages as explained earlier. These grubs upon hatching were reared individually under aseptic condition in plastic containers before inoculating with the bacterium. The stock solution of B. popilliae having 5×10^9 spores/ml was obtained by aseptically bleeding the field collected diseased grubs.

All the experimental grubs of H. consanguinea were surface sterilized using 0.2% sodium hypochlorite. The grubs were first washed thoroughly with tap water to remove soil adhering to their body and then dipped in the sterilizing solution for 5-10 minutes. Traces of sterilizing solution were removed by rinsing the grubs with distilled water.

The grubs were inoculated with the bacterial spores and reared individually in plastic containers (3.5 X 7.5 cm) containing sterilized soil. Optimum level of moisture was maintained in the soil. Washed roots of bajra were provided as food. The food was changed every alternate day. The rearing of the grubs was done at 25 ± 1°C at 86% RH in the laboratory. Percentage of disease was recorded under each method at 7, 14, 21 and 28 days after inoculation. The different inoculation methods tried are as follows :

1. Soil inoculation method :

The required spore concentrations i.e. 1×10^7 and 1×10^9/g of soil, were obtained by mixing 0.8 ml and 80 ml of stock solution respectively in 400 g of sterilized soil. The soil thus inoculated with bacterium was equally distributed in 20 plastic containers and one grub of H. consanguinea was reared in each container. In control, the grubs were reared in sterilized moist soil.

2. Grub gulping :

The grubs were starved for 2 days, prior to inoculation and then dipped in spore suspensions for a minute. The extra suspension on surface was air dried and grubs were reared on bajra roots. In case of control, the grubs were dipped in sterile distilled water for a minute.

3. Root dipping and feeding method :

Seedlings of bajra were washed well with tap water and then with distilled water. The roots were dipped or immersed in a spore suspension having 1×10^7 and 1×10^9 spores/ml for five minutes. After five minutes the roots were taken out and given as food to the grubs. The grubs were fed with treated roots for first five days and thereafter reared on normal bajra roots. Grubs fed on roots which were dipped in sterile water served as control.

4. Injection method :

The grubs were immobilised by keeping them in the deep freezer for 5 minutes and surface sterilized with absolute alcohol. The grubs were injected with 1×10^7 and 1×10^9 spores using sterilized microsyringe and 27 gauze needle. An inoculum of 0.03 ml of spore suspension was injected between second and third posterior abdominal segments of the grubs. Control grubs were injected with 0.03 ml of sterile distilled water. The inoculation

procedure was carried out in the laminar flow chamber to avoid contamination.

5. Soil and root inoculation method :

Soil as well as roots were charged with required spore concentration and grubs were reared as described earlier. The control grubs were reared in sterilized soil and untreated bajra roots were given as food.

6. Inoculation by artificial diet mixed with bacterium :

The artificial diet for P. japonica larvae preposed by Goonewordene (1974) was used with slight modification for the treatment.

(a) Composition of diet :

(i)	Dried bajra seedlings	: 100	g
(ii)	W salt mixture	: 2.5	g
(iii)	Choline chloride	: 1.0	g
(iv)	Linoleic acid	: 1.0	ml
(v)	Vitamins	: 5.0	g

(i) Dried bajra seedings :

A thick sowing of bajra was done in galvanised iron trays. Seven days old seedlings were uprooted and the roots were throughly washed with water to remove soil and then dried. The seedlings were blended for a minute and immersed into acetone to decolourise and soften the tissues. The container having roots dipped

in acetone was kept in fumehood for 3 days and seedlings were then separated from acetone. This material was washed thoroughly under tap water until it became white. The material was then dried in an oven at 40°C for 24 hr. The dried mass was ground and sieved through 80 mesh sieve and stored at 5°C in a refrigerator.

(ii) W salt mixture was obtained from Nutritional Biochemical Corporation, Cleveland, Ohio.

(iii) Linoleic acid 95% was procured from Fluka A.G., Chemiscle, Switzerland.

(iv) Choline Chloride ($C_5H_{14}Cl$) used was the product of Mark, A.G., Dramstadt, Germany.

(v) Vitamins (Multibay) used were manufactured by Bayer (India) Limited, Bombay.

(b) Preparation of diet :

The diet was prepared by grinding weighed quantities of dried bajra seedlings along with other ingredients in a mortar and pestle to get fine powder. Some amount of the diet was mixed with appropriate amount of spore dust to obtain 2×10^7 and 2×10^9 spores/g diet. The diet was stored in a refrigerator.

(c) Rearing of white grub on artificial diet :

The grubs were placed individually in plastic containers (8.8 x 4.5 cm) containing about 150 g of moistened

sterilized soil. About 0.5 g of diet was first spread on moistened Whatman filter paper No. 1 having 8.5 cm diameter and allowed to dry for a few minutes. This filter paper having the diet was placed over the grub. The filter paper was then covered with about 50 g of sterilized moist soil. In experimental sets, contaminated diets were fed to the grubs whereas in control set normal diet was fed to the grubs. The diet was replaced at 7 days interval upto 4 weeks.

Preparation of B. popilliae var. holotrichiae spore powder :

The milky diseased H. consanguinea grubs obtained by artificial infection in the laboratory were crushed in the mixer and resultant material was filtered through cheese cloth to remove debris. The spore strength of the filterate was determined using Neubeaur's chamber under light microscope. The spore suspension was mixed with equal amount of calcium carbonate and mixed thoroughly. The powder obtained by such preparation was dried in shade at room temperature for 24 hr. The powder was stored in the refrigerator at 5 °C.

Method of B. popilliae var. holotrichiae application :

The effectiveness of a pathogen largely depends upon its method of application. Therefore, various methods of application were tried viz., seed treatment with bacterium, furrow application, broadcasting the inoculated soil and

spot application. For this purpose 1 sq m plots were taken for each method and B. popilliae spore powder was applied and groundnut seeds were sown. When the plants were about 3 weeks old, known number of laboratory produced healthy grubs were released and examined after 30 days for the development of disease and percentage of infection due to bacterium. A control set under each method was also maintained. The details of various methods are as under :

Seed coating :

Groundnut seeds were first dipped in spore suspension of B. popilliae having a concentration of 1×10^9 spores/ml. A little amount of arabic gum was also added to the suspension as sticky agent and mixed thoroughly. The seeds were removed after 30 minutes and air dried. The seeds were thus coated with bacterium and used for sowing.

Broadcasting inoculated soil :

About 2 kg of dry soil was taken and spore suspension of B. popilliae having 1×10^9 spore/ml was mixed thoroughly. This spore mixed soil was evenly distributed in plot and mixed in upper 15 cm by digging.

Furrow application :

Furrows were opened and spore powder of B. popilliae was applied at the rate of 10^9 spores/sq m (10^{13} spores/ha).

Spot application :

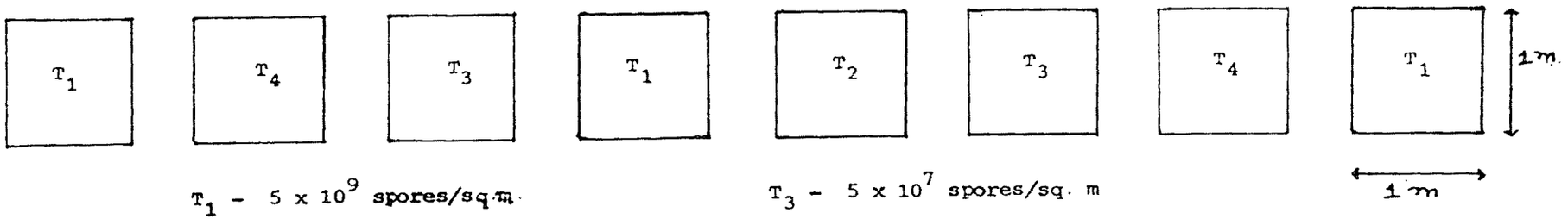
The spore powder of B. popilliae having 10^9 spores/g spore concentration was applied at different spots at the depth of about 15 cm by drilling holes of about 5 mm diameter with the help of iron rod in such a manner that whole area is uniformly covered.

Efficacy of B. popilliae var. holotrichiae against H. consanguinea :

1. Feeler Trial :

A feeler trial was conducted during 1985 season when effectiveness of B. popilliae was tested in microplots (1 x 1 x 0.5 m). The microplots were first washed with water and disinfected with 10% solution of formaldehyde. In plots the basal portion was filled up with gravels up to 30 cm and upper 20 cm with sandyloam type of soil. From the stock solution, 80 ml suspension of 3 different concentrations, i.e. 5×10^9, 5×10^8 and 5×10^7 spores/ml were prepared. The four treatments including control were applied in 4 replications covering a total of 16 microplots. Each treatment was allocated in 4 plots selected at random by means of random number. The lay-out of the treatments is shown in Fig. 2.

Spore suspension (80 ml) of respective concentrations was evenly distributed at different spots by making holes of

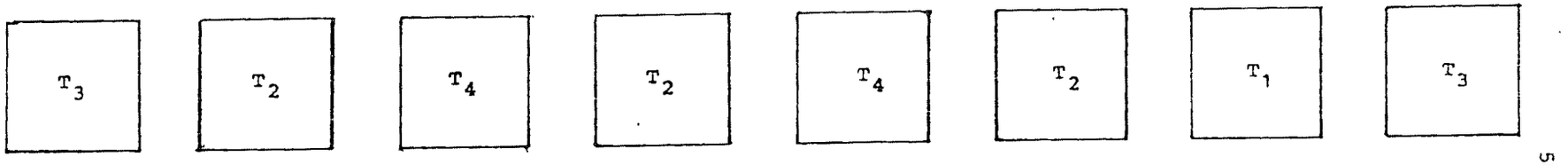


T_1 - 5×10^9 spores/sq.m

T_2 - 5×10^8 spores/sq. m

T_3 - 5×10^7 spores/sq. m

T_4 - Control



about 5 mm diameter at the depth of 15 cm. In case of control, 5 ml sterile distilled water was applied at each spot. Fifty groundnut plants were raised in all the plots as food for white grubs. After 30 days of sowing, twenty 2nd-instar healthy laboratory reared grubs, were released in each plot. The plots were regularly irrigated and weeds were removed. The grubs were recovered after 30 days of release. They were kept in sterilized moist soil and fed on bajra roots in the laboratory for 21 days at 25 $\pm$ 1 °C and 86% RH for further disease development. The mean per cent infection at each concentration was noted from the above observations. The significant difference among the treatments was determined using statistical analysis.

2. Field Trial :

A small scale field trial was conducted at Vatrak, Dist. Sabarkantha during kharif 1986, to test the effectiveness of *B. popilliae* against *H. consanguinea*. The *B. popilliae* spore dust having 1×10^9 spores/g (prepared in the laboratory) was applied in the experimental plots by spot treatment method. In 100 sq m area (7.2 x 14.0 m) total 144 holes each of 1 cm diameter and 15 cm depth were made with a hollow aluminium pipe. About 500 g of *B. popilliae* spore dust was evenly filled in the holes to obtain 5×10^9 spores/sq m (at the rate of 5×10^{13} spores/ha). In control plot only $CaCO_3$ was distributed in same manner. Groundnut

plants raised earlier in two plots with 24 rows in each and having row-to-row distance of 60 cm were chosen. Laboratory reared healthy 2nd-instar H. consanguinea grubs were released in plots to get a population of 3 grubs/sq m. The grubs were recovered at the intervals of 20, 30 and 40 days after the treatment. These grubs were brought to laboratory and reared under aseptic conditions for milky disease development.

Dispersal of B. popilliae var. holotrichiae by birds :

One cattle egret (Babulcus ibis), two myna (Acridotheres tristis) and four house sparrows (Passer domesticus) were captured from the field. These birds were brought to the laboratory and kept individually in wooden cages (45 x 45 x 45 cm) fitted with wire mesh sides and glass ceiling. The bottom of each cage was lined with polythene sheet which facilitated collection of excretal droppings. The experimental birds, myna (1) sparrow (3) and egret (1) were fed respectively according to their body weight with 5.0, 3.0 and 10.0 grams of grubs infected with B. popilliae. One bird was kept as control in case of myna and sparrow, however, no control could be maintained in case of egret. Control myna was fed with sorghum grains and ripe tomatoes while sparrow was fed with other small cereal grains.

The droppings of experimental and control birds were collected everyday, dried, weighed and crushed in a mortar

and pestle to get fine powder. This powder was microscopically examined to see the presence of bacterial spores and spores were counted using Neubeaur's chamber. The dropping powder was mixed with sterilized soil (1:3 ratio) and used for rearing healthy white grubs to conduct pathogenicity tests. Since quantity of excretal droppings of experimental birds was very meagre, only 12 laboratory reared healthy grubs could be tested for pathogenicity. However, pathogenicity could not be tested in case of egret where only microscopic examination was done. Similarly 12 laboratory reared healthy grubs were tested against control bird's droppings.

Studies on Beauveria brongniartii :

B. brongniartii (saccordo) Petch (= B. tenella) a white muscardine fungus has been recorded as a promising pathogen of H. serrata in Bangalore. With a view to introduce this fungal agent on Holotrichia spp. in Gujarat, studies were carried out. The nucleus culture of the fungus was obtained through the courtesy of Dr. G.K. Veeresh, University of Agriculture Science, Bangalore, Karnataka.

Growth and sporulation of B. brongniartii on various laboratory media :

Various solid and liquid media were tested for their efficacy for the growth and sporulation of the fungus in

the laboratory. The media used were Potato dextrose agar (Anon., 1948), Sabouraud dextrose agar (Sabouraud, 1893), Sabouraud maltose agar (Sabouraud, 1893), Sabouraud maltose agar + 1% yeast (Theone and Joseph, 1962), Rice flour agar and Maize flour agar. The composition of media are as follows :

Potato dextrose agar :

Potatoes infusion	:	200	g
Dextrose	:	20	g
Agar	:	15	g
Distilled water	:	1000	ml

pH - 4.5

Sabouraud dextrose agar :

Peptone	:	10	g
Dextrose	:	40	g
Agar	:	15	g
Distilled water	:	1000	ml

pH - 5.6

Sabouraud maltose agar :

Peptone	:	10	g
Maltose	:	40	g
Agar	:	15	g
Distilled water	:	1000	ml

pH - 5.6

Sabouraud maltose agar + 1 % yeast :

Peptone	:	10 g
Yeast extract	:	10 g
Maltose	:	40 g
Agar	:	15 g
Distilled water	:	1000 ml

pH - 5.6

Rice flour agar :

Rice flour	:	40 g
Agar	:	15 g
Distilled water	:	1000 ml

pH - 5.6

Maize flour agar :

Maize flour	:	40 g
Agar	:	15 g
Distilled water	:	1000 ml

pH - 5.6

Broths of all the above media had the same formula except agar is omitted. In case of broths, 50 ml of medium was taken in each of 3 conical flasks of 100 ml capacity, making a total of 18 flasks for 6 different media. Side by side 50 ml of solid media in each case of 6 different media were prepared in 100 ml capacity flasks. All were autoclaved alongwith 18 petriplates of 8 cm diameter at 121 °C for

15 minutes. In case of solid media, 50 ml was poured in to each of 3 petriplates after autoclaving, totalling 18 plates for 6 different media. After cooling, the broths were inoculated with 0.1 ml of B. brongniartii conidial suspension having 10^7 conidia/ml. On solid media, 0.1 ml of suspension was inoculated using sterile spreader. Plates and flasks were incubated in BOD incubator at 26 ± 1°C for 10 days.

The sporulation on respective media was measured after 10 days of growth. From each plate 10 mm block was taken using sterile borer. The block was suspended in 10 ml of 0.1% sterile tween - 80 solution and mixed thoroughly by crushing with a sterile glass rod. The conidia were counted using Neubeaur's chamber. From the flasks the fungal mass was harvested by filteration through Whatman filter paper and wet weight of mycelial mass was recorded. The material was dried in oven at 40°C for 24 hr. The dry mycelial weight was also recorded.

Pathogenicity of B. brongniartii against different stages of important species of white grub :

Pathogenicity of B. brongniartii was determined to egg, larvae, pupa and adult of different species of white grubs viz., H. consanguinea, H. serrata, H. fregei and A. nathani in the laboratory. Appropriate amount of fungal

conidia were mixed in sterilized moist soil to obtain 10^7 conidia/g. The conidia were obtained by harvesting B. brongniartii from Sabouraud maltose agar + 1% yeast medium, having 8-10 days growth. The laboratory reared 2nd-instar grubs of each species were individually reared in this infected soil. The grubs were fed on bajra roots and development of mycosis was observed.

In case of adults, 5 kg of moist soil having 10^7 conidia/g was taken in round galvanized cages (35 cm diameter and 10 cm high) and 25 adults of each species were released in respective cages. Freshly formed pupae and eggs of each species were taken in different petridishes (10 cm) having blotting paper on the bottom. The pupae and eggs were sprayed with suspension having 10^7 conidia/ml by glass sprayer of 50 ml capacity. The pupae and eggs were then transferred into plastic container (8.5 x 4.5 cm) having sterilized moist soil and observed for development of mycosis. The eggs were observed till hatching and pupa till it transformed into adult stage.

Pathogenicity of B. brongniartii to some destructive insect pests :

The major insect pests commonly found in this area were tested for their susceptibility to B. brongniartii. Laboratory reared larvae of gram pod borer Heliothis armigera, H. peltigera, castor semilooper Achaeae janata, cabbage

semilooper Trichoplusia ni., tobacco leaf eating caterpillar Sopodoptera litura, S. exiguae, cut worms Agrotis ipsilon, A. segetum and A. spinifera, Gujarat hairy caterpillar Amsacta moorei, rice moth Corcyra cephalonica and medicinal plant pest Catopsilia pyranthe were collected from the laboratory cultures of All India Co-ordinated Research Project on Biological Control of Crop Pests and Weeds, Gujarat Agricultural University, Anand.

Twenty-five healthy larvae of each insect were tested for the pathogenicity of B. brongniartii by tropical application of fungus in the laboratory. The larvae were allowed to crawl on 10 days old fungal growth on SMY agar. The larvae were crawled for 15 min with gentle shaking of petriplate for proper contamination with the fungal conidia. After treatment they were reared individually on their preferred host plant leaves in plastic container(2.5 x 7.5 cm). All the containers were kept at 25 $\pm$ 1 $^{\circ}$C in the laboratory. Larvae were observed daily for the fungal infection.

Mass production of B. brongniartii in the laboratory :

Various cereal grains viz., wheat, paddy, maize, rajagira, pearl millet, great millet and kodo millet were tested as suitable solid substrate for the growth of B. brongniartii. The cereals were crushed in the household mixer for 30 seconds to make small pieces. The 50 g of broken grains were taken in 250 ml flasks and 45 ml water

was added to each flask. The grains were sterilized by autoclaving at 121°C for 30 min. After cooling, each flask was inoculated with 5 ml of B. brongniartii spore suspension (1×10^6 spores/ml). The flasks were incubated at 26 ± 1°C for 15-20 days.

In case of maize grains, the germinated grains were also tested. Maize grains were first soacked in water for 24 hr. The grains were then allowed to germinate on wet blotting paper sheet. The germinated grains were crushed in mixer and used as substrate like other grains described above.

Some agricultural waste materials were also tested for the growth and sporulation of B. brongniartii. Husk and straw of wheat and rice, treated with alkali and acid or untreated were tested. The waste obtained from cattle feed industries mainly constitute of castor seeds was also tested. About 25 g of above materials were mixed with 10 ml water in 250 ml flasks. The flasks were autoclaved for 30 mins at 121°C. The sterile flasks were inoculated after cooling with 5 ml of B. brongniartii spore suspension. They were further incubated for growth and sporulation of fungus in the laboratory.

The fully grown and sporulated fungal mass from each flasks was taken out after 20 days. The material was

crushed after drying and spore load of B. brongniartii from all the samples was determined.

Mass production of B. brongniartii conidia on maize in autoclavable plastic bags and galvanized iron trays :

From all the cereals tested, maize was found best and mass production of fungus was done using maize as a substrate. High density polyethylene (HDPE) bags procured from Vallabh Polypac Industries Pvt. Ltd., Vitthal Udyognagar, Anand, Gujarat, were used for mass production. About 100 g of broken maize grains were taken in bags (23 x 17 cm) and 95 ml water was added to it. The bags were then sealed on gas burner flame. The bags were autoclaved at 121 °C for 45 minutes and cooled down to room temperature. One corner of the bags was cut with a sterilized scissor under aseptic condition. From this open area, 5 ml of B. brongniartii conidial suspension (1 x 10^6 conidia/ml) was inoculated using sterile pipettes. The open area was resealed on the flame. The bags were incubated at 26 ± 1 °C for 20 days.

In galvanised iron sheet trays (45 x 20 x 10 cm) 250 g of crushed maize grains were mixed with 240 ml of water. Trays were covered with aluminium foil and fitted at the edge of tray with the help of string. The trays were autoclaved for 45 min at 121 °C. After cooling, the maize grain pads in trays were inoculated with 20 ml of conidial suspension having 10^6 conidia/ml using sterilized pipettes

from corners under aseptic conditions. Trays were incubated at 26 ± 1 °C for 20 days.

The fungal mass was harvested along with maize from bags and trays, and dried at 40 °C for 24 hr. Dried mass was ground along with grain carrier to get fine powder.

Field efficacy of B. brongniartii against white grubs :

A small scale field trial was conducted to test the effectiveness of B. brongniartii against H. serrata at Anand, during 1986. In 100 sq ft (18 sq m) plot the B. brongniartii spore dust was applied at the rate of 10^{15} conidia/ha. Total 4 kg B. brongniartii spore powder along with grain carrier was applied by furrow treatment before sowing. Untreated plot of same size was also kept as control. In both the plots groundnut crop was sown during June.

After one month of plant growth, 100 laboratory reared 2nd instar H. serrata grubs were released in both the plots, to get 1 grub/sq ft population. Grubs were collected after 3rd, 4th and 5th week of release from the plots. The collected grubs were further reared in the laboratory under aseptic conditions to observe and record the white muscardine disease.

Similarly, another field trial was conducted at Anand during 1987, in which the effectiveness of B. brongniartii was checked against H. consanguinea. All the parameters were kept same as those described in earlier testing.

A field trial was also conducted during 1987 at Vijapur, Dist. Mehsana. The B. brongniartii spore dust was applied at the rate of 10^{15} conidia/ha in 100 sq m area. The spores were applied in furrows before sowing. Groundnut crop was sown in the plot. A untreated area of 100 sq m was also maintained at the same place which served as control. The grubs were periodically collected from experimental as well as control plots and reared further under aseptic conditions to record percentage mortality due to fungus.

Compatibility of B. brongniartii with some pesticides and B. popilliae :

Compatibility with pesticides :

Insecticides and fungicides commonly used in groundnut crop were tested for their compatibility with B. brongniartii by poisoned food diet technique. The insecticides tested were lindane, phorate, sevidol, carbofuran and quinalphos, whereas fungicides were fytolane, thiram, captan, bavistin and mancozeb. The insecticides were tested at 3 different doses, field recommended (1X), half the field recommended (½X) and double the field recommended dose (2X). Aqueous solution of Sabouraud maltose agar + 1% yeast was prepared and 100 ml of medium was dispensed in 250 ml flasks. The appropriate amount of insecticides was mixed in respective media to obtain X, 2X and ½X concentrations. The flasks were sterilized at 121°C for 15 min and allowed to cool

till 45°C. The 100 ml medium of each flask was equally distributed in 5 petriplates having 8.0 cm diameter, which made a final area of 251 sq cm. Similarly 3 different concentrations, 100, 500 and 1000 ppm of fungicides were obtained by mixing the appropriate amount of respective fungicides in SMYA. The SMYA without pesticides served as control.

Small blocks of 1.0 cm diameter of actively growing B. brongniartii were scalped out from SMYA using cork borer. The blocks were transferred to insecticide and fungicide treated plates in aseptic conditions. The petriplates were incubated at 26 ± 1°C in BOD incubator. The observations on fungal growth were made after 6, 8 and 10 days. The diameter of growth zone was measured in treated and untreated plates. Per cent growth inhibition was calculated by the following formula given by Vincent (1927).

$$\text{Percentage inhibition} = \frac{100 \times C - T}{C}$$

where, C = Average diameter of growth in control set,

T = Average diameter of growth in experimental set.

Extent of sporulation was measured by taking 10 mm block of 10 days old fungal growth from each plate. The block was suspended in sterile 0.1% tween-80 solution and mixed thoroughly. Spore count was done from this suspension using Neubeaur's chamber.

Compatibility with B. popilliae var. holotrichiae :

Twenty five healthy H. consanguinea grubs were reared in soil having 10^7 B. popilliae spores/g for one week. Afterwards, the grubs were reared in soil having 10^7 B. brongniartii conidia/g for one week. Those grubs were further reared in sterile moist soil for one month and regularly observed for disease development by the pathogens.

In another set, B. popilliae and B. brongniartii spores were added to sterilized soil to obtain 10^7 spores/g of each. This soil was distributed in 25 plastic containers and 25 healthy grubs were reared individually in it. All the containers were kept at 26 $\pm$ 1 $^{\circ}$C in the laboratory. The disease incidence in the grubs by both the pathogens was recorded.