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Household Production of *Isaria fumosorosea* Fungus Mother Culture to Control Rugose Spiraling Whitefly

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Rugose spiraling whitefly (RSW), Aleurodicus rugioperculatus *Martin. is an invasive pest in India. It feeds on most of the green-leaved plants including arecaceae palms. Both the nymphs and adults suck the sap from the leaves causing yield loss. For effective management of the pest, ICAR- Indian Institute of Oil Palm Research, Pedavegi has developed a novel technology using microbial agent,* Isaria fumosorosea. *The initial trials that were conducted on the efficacy of the fungus yielded good results causing substantial mortality to all stages of the pest. The methodology on multiplication has been standardised using locally available low cost agro materials like broken sago grains, cane sugar and potatoes. Multiplication of the microbial organism included preparation of mother culture in the laboratory as well as in the farmers' homes and preparation of ready to use culture for application against the target pest using mother culture. The preparation of mother culture using laboratory ingredients was compared with that of locally available materials. The spore count and the time taken for obtaining the maximum output were also compared and found on par in both the cases. The methodology to multiply the mother culture by farmers in their homes was also developed which is helpful for further multiplication and use to control the pest. The cost of production of mother culture using locally available agro materials was found to be half the price of that prepared in the laboratory. This methodology replaces the farmers dependency for mother culture from various institutes/laboratories and helps to procure sufficient culture with them.*

Keywords*: Rugose spiraling whitefly,* Isaria fumosorosea*, broken sago grains, cane sugar*

Oil palm is an introduced crop to India. It is grown as irrigated crop in majority of the potential states of the country. In recent years the crop is being infested with an invasive pest, rugose spiraling whitefly (RSW), *Aleurodicus rugioperculatus* Martin (Anon, 2018; Kalidas, 2018). The increasing incidence of the pest is leading to growth and yield loss (Sundararaj & Selvaraj, 2017; Kalidas, 2019) in not only arecaceae plants but also on other horticultural crops including nursery plants (*Figure 1*).

The integrated pest management practices that were developed to manage the pest include hanging of yellow sticky traps, growing French marigold plants, release of locally available natural enemies *viz. Encarsia guadeloupae* and *Dichocrysa astur* and application of *Isaria fumosorosea* fungus mixed with detergent powder. Of these, the entomopathogenic fungus, *Isaria fumosorosea* (*Figure 2*) is found to suppress the pest effectively (Kalidas, 2020; Vivek Kumar *et al.*, 2016).

The fungus is an effective microbial organism that develops and multiplies on the whiteflies worldwide causing yellow muscardine disease leading to mortality of the

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Oil palm *Coconut seedling* *Coconut adult palm*

Arecaceae palms

Soursop *Indian gooseberry/Emblic* *Guava* *Heliconia*

Horticultural plants

Figure 1 Incidence of rugose spiraling whitefly on arecaceae palms and horticultural plants

Figure 2 Isaria fumosorosea *fungus on PDA*

pest (Sandhu *et al*., 2012; Zimmerman, 2008) (*Figure 3*). Research results by Vivek Kumar *et al*. (2016) from University of Florida, USA confirmed the efficacy of the fungus against the pest. Work was also carried out at ICAR-IIOPR, Pedavegi using locally available strain of the fungus and was found to be effective causing heavy mortality to the pest. Gabriel *et al*. (2010) proved the importance of agro industrial resources for the rapid production of the fungus, *Isaria fumosorosea*, with higher yield of conidia. In the present study, methodologies have been developed for the production of the fungus, *Isaria fumosorosea*

Figure 3 Isaria *fungus infection on rugose spiraling whitefly adults*

using locally available agro materials in a cost effective manner so that farmers can produce the fungus in their homes with the help of their family members. The present work relates to the steps followed in achieving the same.

MATERIALS AND METHODS

Production of mother culture

The *Isaria fumosorosea* strain was multiplied on potato dextrose agar (PDA) media in petriplates using standard procedure. After obtaining sufficient culture, multiplication of liquid formulation of fungus was carried out using the following equipment and materials (*Figure 4*).

- Plastic trays of 30 x 40 x 6 cm size,
- Surgical spirit,
- Surgical cotton,
- Starch powder (broken sago grains),
- Streptomycin antibiotic powder,
- Gift wrapping paper roll,
- Rubber bands of big size (to cover the trays with gift paper) and small size (to cover the conical flask/glass saline bottle mouth with paper),
- Potato dextrose broth (PDB),
- Inoculation loops/ stainless steel spoon/ forceps,
- Conical flask/ saline glass bottle,
- Gas stove,
- Pressure cooker,
- Candle light,
- Potatoes,
- Bunsen burner,
- Label sticker,
- Power mixer grinder,
- Mask, and
- Surgical gloves.

Methodology followed

Preparation of mother culture in laboratory

The culture medium to grow fungus was prepared using starch powder (broken sago grains) and PDB. Three treatments were tested wherein both the ingredients were mixed at :

i) 7.5 g starch +7.5 g PDB
ii) 5 g starch + 10 g PDB
iii) 10 g starch + 5 g PDB

Both the ingredients were poured into conical flasks containing 1000 ml of distilled water. The mouth of the conical flasks were closed with cotton plugs which were further covered with metallic colour paper wrapping sheets and tightened with rubber bands. These conical flasks were kept in autoclave and sterilised at 6.80 kg pressure. The sterilised media are ready to be poured into cleaned and air dried plastice trays. Prior to pouring the media, the trays were again cleaned with surgical spirit. Necessary precautions like wearing hand gloves and nose mask, sterilising the hands using spirit were followed while doing the media preparation and inoculation. The

Plastic tray *Surgical spirit* *Surgical cotton* *Starch/broken sago grains*

Streptomycin antibiotic *Gift paper roll* *Rubber bands* *Potato dextrose broth*

Inoculation loop *Forceps* *Spoon* *Conical flask* *Saline bottles* *Stove and cooker*

Wax candle *Potatoes* *Bunsen burner* *Label stickers*

Power mixer grinder *Mask* *Surgical gloves*

Figure 4 Equipment and materials used

cooled prepared media was poured into the plastic trays and 100 mg of streptomycin antibiotic was added to arrest the bacterial contamination. Later, using an inoculation loop, the prepared fungus culture of *Isaria fumosorosea* was transferred from petriplates to the plastic trays. Three pieces of 1 sq cm size each of the fungus culture were added to the media. After completion of transfer, the media was disturbed using the same loop for uniform distribution of spores. The tray was then covered with gift paper using big rubber band. A sticker was pasted over the tray indicating the date of inoculation, etc. All the above operations were done in the inoculation chamber in the presence of a Bunsen burner. These inoculated trays were piled in temperature-controlled room on steel racks. Care was taken while maintaining the room temperature at 26°C. Similarly, care was also taken for maintaining cleanliness and hygienic conditions in the growth chamber as well as inoculation rooms (*Figure 5*).

Preparation of mother culture by farmers in their homes

To multiply the fungus mother culture by farmers in their homes, the following methodology was tested using the locally available and cost-effective ingredients.

i) Potatoes in place of PDB,
ii) Forceps/stainless steel spoon in place of inoculation loop, and
iii) Used glass saline bottles in place of conical flasks.

Two treatments were tried which include use of potatoes at 20 g and 40 g. The potatoes were boiled in a pressure cooker and the skin was peeled. They were then macerated into semi-solid material using a power mixer grinder. To these, 5 g of starch was added and both the treatments were put in saline bottles containing 1000 ml distilled or Reverse Osmosis water (RO). The rest of the procedure followed was similar to that done in laboratory production. The inoculation was done using forceps/ stainless steel spoon instead of inoculation loop. In place of Bunsen burner, candle light was used to prevent the contamination (*Figure 6*).

Preparation of ready to use culture by planters

Using the mother culture obtained from laboratories/households, the ready to use culture was prepared with locally available cost-effective materials. The methodology developed was suitable for the farmers to prepare the material by themselves in their own farm under shade. The following ingredients were used as input material.

i) One hundred litres capacity plastic or cement container,
ii) Cane sugar,
iii) Starch (broken sago grains),
iv) Bamboo stick/rod, and
v) Porous cloth.

A large container was filled with 100 L of water and 2 kg of cane sugar was added to dissolve in it. To this solution, 2 kg of boiled starch was added. To keep the starch free from clumps, continuous stirring was done while boiling. Mother culture of *Isaria fumosorosea* (500 ml) was added to this mixture. The container was covered with a porous cloth to arrest the entry of unwanted things like insects, dust particles etc. The above material was stirred daily at frequent intervals by using a bamboo pole/rod. The procedure is to provide air to the spores as they are aerobic fungi. After one week, the spores were counted using Neubauer hemocytometer.

Potato dextrose broth

Broken sago grains

Ingredients in conical flask with cotton plug

Placing conical flask in autoclave

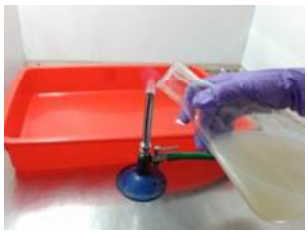
Sterilisation of ingredients in autoclave at 6.80 kg pressure

Cooling of sterilised ingredients

Plastic trays

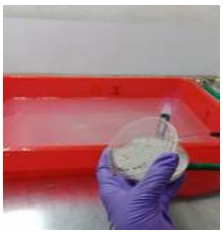
Surgical spirit

Cleaning trays with surgical spirit

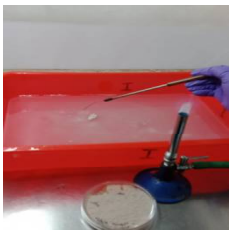


Pouring media into trays in the presence of flame

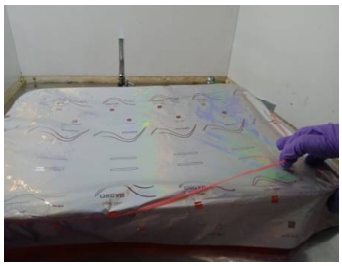
Addition of antibiotic



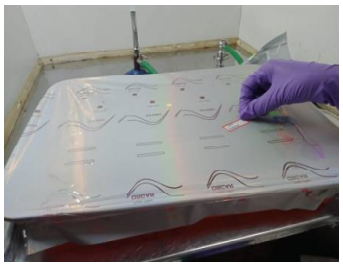
Transfer of fungus culture



Mixing of culture into media using inoculation loop



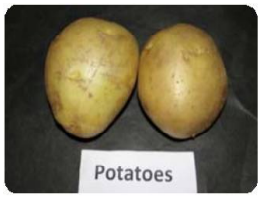
Covering the tray with gift paper and rubber band



Affixing label sticker

Piling of inoculated trays

Figure 5 Multiplication of liquid mother culture in laboratory



Potatoes


Cut potato pieces


Boiling in pressure cooker


Peeled potatoes after boiling


Grinding peeled potatoes


Macerated potatoes

Boiling starch

Mixture of macerated potatoes and starch


Pouring the mixture into saline bottle


Closing the bottle mouth with cotton plug as lid


Covering the lid with paper and rubber band


Sterilising the media in pressure cooker


Pouring the sterilised media into tray in the presence of candle flame


Adding antibiotic


Burning the stainless steel spoon over candle flame


Removing culture pieces from petri plate using spoon


Inoculation of media


Covering the inoculated tray using gift paper and rubber band


Affixing the label sticker


Piling of inoculated trays


Culture growth after 5 days of inoculation


Culture growth after 12 days of inoculation


Culture ready for grinding


Final product of mother culture

Figure 6 Multiplication of Isaria fumosorosea *mother culture in farmer's house*

RESULTS AND DISCUSSION

Preparation in laboratory

In both, laboratory as well as the farmer's preparations, the fungus culture started multiplying from the second day onwards with the appearance of white coloured masses (*Figure 7*) and occupied the entire tray by the fifth day after inoculation (*Figure 8*). By the twelfth day, the white colour mat turned grey (*Figure 9*) indicating the readiness of the culture. This was further confirmed by taking the spore count using Neubauer hemocytometer. A spore count of 160 -190 X 10^8 was obtained from these trays. After 12 days of inoculation, the fully grown culture in the trays was thoroughly mixed using a power mixer grinder, and then transferred to 20 L containers and

Figure 7 Two days old Isaria fumosorosea *culture*

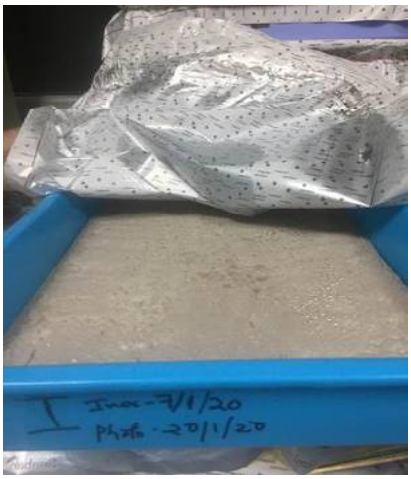


Figure 9 Twelve days old Isaria fumosorosea

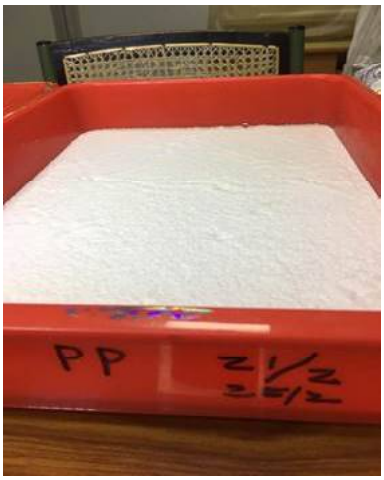


Figure 8 Five days old Isaria fumosorosea *culture*

Figure 10 Isaria fumosorosea *mother culture*

stored as mother culture (*Figure 10*). This was then transferred to half litre bottles and used for distribution to needy planters for further multiplication to obtain ready to use culture. It was found that the spore count in the mother culture was more (227 x 10^8) when the same quantity of both starch and PDB ingredients were used i.e. 7.5 g + 7.5 g compared to only starch (*Table 1*). Although both the treatments containing PDB as ingredient recorded same quantity of spores, considering the cost of PDB, this treatment is adjudged as the best and effective to use.

Preparation at farmer's house

The growth of the culture as well as its spore count was calculated and compared with that prepared in the laboratory. It was found that potatoes used at 40 g proved effective with a spore count of 178 x 10^8 compared to 20 g (100 x 10^8) and was on par with the laboratory cultured one.

The cost of preparation of culture with potatoes as main ingredient was found to be half the amount compared to laboratory method. This was mainly due to the high cost of PDB. By taking all the expenses into consideration, the cost of preparation with potatoes was found to be Rs. 69 (=1 US $) per litre compared to Rs 126 (=1.82 US $) in case of lab cultured one. The culture can thus be prepared by individual farmers in their own house utilising the services of their family members as and when required (*Table 2*).

Preparation of ready to use culture

It is observed that bubbles started appearing on the water surface from the third day onwards indicating that the fungus is multiplying. After one week, the spores were counted in this prepared solution using Neubauer hemocytometer. The count was observed to be in the range of 170 - 200 x 10^8 per mililitre thus indicating that the material is ready to use. In those containers where the required spore count of 170 - 200 x 10^8 per mililitre was not obtained, the stirring was continued for a few more days. When the count was not optimum or the material is not for immediate use, one more application of boiled starch yielded good results as it acts as food for the already developed spores.

Application of ready to use culture against the pest

The ready to use *Isaria fumosorosea* fungus culture that was prepared using mother culture solution was recommended to be used at a concentration of 5 ml per every litre of water. To this, addition of 5 g of detergent powder

TABLE 1
SPORE COUNT (..X 10^8) IN DIFFERENT TREATMENTS

No.	*Starch (30 g)*	*Starch+PDB (7.5g+7.5g)*	*Starch+PDB (5g+10g)*
1	122	218	198
2	140	252	223
3	206	213	260
Average	156	227	227

Note: PDB – potato dextrose broth

TABLE 2
COST OF PREPARATION OF 1 LITRE MEDIUM

		Farmer's house		*Laboratory*	
No	*Item*	*Amount used*	*Price (Rs.)*	*Amount used*	*Price (Rs.)*
1	Potatoes	40 g	0.8	7.5 g (PDB)	58.9
2	Starch	5 g	0.2	7.5 g	0.3
3	Streptomycin	0.13 g	1.25	0.13 g	1.25
4	Spirit	3ml	0.24	3ml	0.24
5	Cotton	10 g	4.8	10 g	4.8
6	Covering sheet	1.5m	0.24	1.5m	0.24
7	Rubber band	2	0.5	2	0.5
8	Gas	2 h	12.5	2 h	12.5
9	Mask	1	0.12	1	0.12
10	Hand gloves	2	0.672	2	0.672
11	Bottles	2	5.6	2	5.6
12	Salary (technical)	1 L	20	1 L	20.0
13	Salary (non technical)	1 L	13.32	1 L	13.32
14	Microscopic cover glass	1	0.965	1	0.965
15	Bottles stickers	2	4.4	2	4.4
16	Other miscellaneous	Electricity, water etc.	3	Electricity, water etc.	3
	Total		68.607		126.807

Note: PDB – potato dextrose broth; 1USD = Rs69

was also recommended just before spraying. Since the pest is covered with wax floccus material, the detergent powder was found to be effective in dissolving the wax and exposed the pest. This helped the fungus to work by penetrating through the cuticle of the pest. Since the fungus is a slow growing one, multiple applications were found essential with a minimum of three sprays. Thorough coverage of the plant (without leaving any leaf uncovered) particularly on the under surface of the canopy was found effective and hence found essential to obtain successful control of the pest. There was no sign of attack of the treated leaves by the pest even up to 9 months of application indicating that the spores are active till that period.

CONCLUSION

Use of microbial organism, *Isaria fumosorosea* has become a critical tool in the management of the invasive rugose spiraling whitefly as it is found to be effective in killing the pest population. Based on the results obtained in different experiments conducted on various plants, it is reported that the fungus is very effective in managing the pest population. So far the mother culture of the fungus is available from laboratories and are being supplied to the farmers. To avoid the rush as well as narrow down the time between demand and supply of the culture, the present work was carried out to multiply the fungus mother culture by farmers themselves in their own homes. Using

locally available material, the multiplication has been made easy and simple. The cost of multiplication has been reduced to half the price to that of laboratory multiplied ones. The spore count was found to be the same in both the cultures indicating that the technology is cost effective and can be multiplied by all the farmers in their homes. By doing this, farmers can have sufficient mother culture with them to use against the pest population. The methodology is also found to be effective in multiplying other bio microbes like *Trichoderma* and *Metarhizium* spp. which are commonly used in plantations. Hence it is found useful to small scale farmers of all the oil palm growing countries.

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