

# COMPENDIUM OF COTTON MEALYBUGS

V. S. Nagrare, Sandhya Kranthi, Rishi Kumar, B. Dhara Jothi, M. Amutha, A. J. Deshmukh, K. D. Bisane and K. R. Kranthi



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Research into Decision Support System for Management of Insect Pests of Major Rice and Cotton Based Cropping Systems



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#### COMPENDIUM OF COTTON MEALYBUGS



#### प्रो.स वपनकुमारद त्ता उपमहानिदेशक( फसलविज्ञान)

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# FOREWORD

Changes in pest scenario have become more common in the recent times in the rapidly changing cropping systems and environment with the dominance of Bt cotton across India. Some cotton pests have been impacted negatively while some of the pests which were not known earlier have emerged as major pests. An introduced species of mealybug, *Phenococus solenopsis* Tinsley caused havoc in all 9 cotton growing states of the country causing heavy losses up to 40-50% of affected fields during 2007-08. Being an introduced exotic pest, the knowledge among the farmers and researchers was lacking. Central Institute for Cotton Research, Nagpur has taken timely steps in initiating basic studies related to correct identification of the species, seasonal occurrence, host range, biology, molecular diversity, natural enemies and other related aspects in the crop production environment which have direct relevance to the formulation of effective management strategies in order to discourage indiscriminate use of hazardous chemicals.

Information on mealybugs has been published as research papers, advisory folders, leaflets, posters and videos from government agencies, agricultural universities etc with limited coverage. However comprehensive information on various aspects of mealybugs has been lacking.

With the widespread occurrence of mealybugs during 2007, the National Agricultural Innovative Project (NAIP) considered mealybug as one of the major problems and developed a frame work on development of Decision Support System for management of insect pests of cotton for studies of diversity and biology, besides quantification of host range and natural control by natural enemies. The launching of the project in 2008 and funding through World Bank, assisted greatly in speeding up research on mealybugs.

During the last 2-3 years substantial data have been generated on biology, host range, natural enemies, effect of temperature on the development of mealybugs etc. During the course of studies it was observed that *P. solenopsis* being a polyphagous pest and sessile in nature, thus promoting studies on weed management and conservation of natural enemies for keeping the populations of *P. solenopsis* under check. Among the different species of natural enemies, species specific parasitoid *Aenasius bambawalei*, has a major role in the natural control of mealybug. During these years it was felt that conservation of natural enemies is the need of the hour before resorting to the chemicals. "Compendium of Cotton Mealybugs" presents the details of species identification characters, seasonal occurrence and severity, spread and host range, biology suggesting the necessary management strategies that would prove useful for plant protection scientist, farmers, researchers, planners and students alike. The research team of the CICR especially Dr. V. S. Nagrare and colleagues deserve commendation in bringing out this publication for all interested users.

S. W. Ste

(Swapan Kumar Datta)

# ACKNOWLEDGMENTS

It gives indeed a great pleasure to be associated with research work on a challenging pest, mealybugs, when every entomologist of the country was unaware of the species identity that spread across the country within short time span. The opportunity was fully utilized and knowledge on mealybug diversity, distribution, abundance, host range, biology and influence of natural biotic and abiotic forces in the environment to the fullest extent was aquired. I am privileged to be associated with mealybugs since 2007 in the institute funded project "Studies on bionomics and management of mealybugs" earlier and World Bank funded project thereafter to understand the potential of mealybugs as pests of cotton and immediate steps to be taken to limit its spread and severity. The vision of National Agricultural Innovation Project (NAIP) advisory committee members Dr. T. M. Manjunath and Dr. S. Lingappa to recommend mealybugs as one of the target insects under the project on "Decision Support System for the Management of Insect Pests of Major Rice and Cotton based Cropping Systems" allowed us to use the resources of time, manpower and money especially for mealybugs. Consortium Principal Investigator Dr. Y. G. Prasad, Principal Scientist, Central Research Institute for Dryland Agriculture, Hyderabad deserves our sincere appreciation for the constant encouragement for bringing out the compendium to serve as a base line for further studies on mealybug that will be helpful in fine tuning management strategies. I am extremely grateful to Dr. K. R. Kranthi, Director, Central Institute for Cotton Research, Nagpur who, being an entomologist with visionary approach recommended "not to resort to chemical sprays on young plants that have slight infestation of the mealybugs in early vegetative stages of the crop" and this worked well. It saved crores of rupees by way of using minimal chemicals and also helped in conserving ecosystem. His personal interest, constant guidance and suggestions on the mealybug studies showed new direction in the management of this new exotic pest on cotton. The research work furnished in the bulletin was carried under the able guidance of Dr. K. R. Kranthi at CICR Nagpur and its regional stations at Sirsa and Coimbatore. Our sincere thanks are due to Shri M. K. Meshram, Principal Scientist and in charge Head, Division of Crop Protection, CICR, Nagpur, Dr. D. Monga, Head, CICR, RS Sirsa and Dr. N. Gopalakrishnan CICR RS Coimbatore for providing basic research facilities required at respective stations. The identification of parasitoid Aenasius bambawalei by Dr. M. Hayat is duly acknowledged. The identification services of mealybugs and their parasitoids provided by Dr. V.V. Ramamurthy and S. Joshi of IARI, New Delhi and of host plant species by Dr. P.C. Pagar, College of Agriculture (PDKV), Nagpur are acknowledged. The genuine and tireless assistance offered by D. S. Bhongle, J. L. Dongre, Asmita, Ranjana and Sanjay is duly recognized with gratitude. I thankfully acknowledge the financial assistance by World Bank through Indian Council of Agricultural Research, New Delhi to carry out the present study as a part of National Agricultural Innovation Project (NAIP/DSS/C 2046) at Central Institute for Cotton Research, Nagpur.

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COMPENDIUM OF COTTON MEALYBUGS

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#### **1. INTRODUCTION**



Cotton is an important natural fibre crop cultivated in varying climatic conditions of tropics as well sub-tropic regions of more than 83 countries all over the world. Cotton plays a key role in the National economy in terms of generation of direct and indirect employment in the Agricultural and Industrial sectors. Due to ready availability of Bt-cotton seeds since 2002 and apperent advantages over non-Bt counterparts, it spread rapidly in India within short span of time. Changes in insect pest complex were evident with changed micro-climate. A new pest, mealybug which was hitherto not familiar earlier started destroying cotton crops caused economic damage, reducing yields up to 40-50% in affected fields since

2006. Several parts of Gujarat which are located on the border of Pakistan which had recent history of mealybugs infestation was severely affected. Mealybug infestation were recorded (Dhara Jothi et al., 2008) in 2006 on G. hirsutum in all the nine cotton-growing states of India, Punjab, Haryana, Rajasthan, Gujarat, Madhya Pradesh, Maharashtra, Tamil Nadu, Andhra Pradesh and Karnataka. Severe economic damage to G. hirsutum was reported in 2007 (Dhara Jothi et al., 2008) in four major cotton-growing districts (Bathinda, Muktsar, Faridkot and Ferozepur) of Punjab, two districts (Hisar and Sirsa) of Haryana, and low to moderate damage in parts of Maharashtra, Tamil Nadu and Andhra Pradesh. According to Goswami (2007), nearly 2000 acres of cotton crops were destroyed by the mealybug by mid-July and over 100 acres of mealybug-infested Bt cotton was uprooted in Raike-Kalan village in Bathinda. By the end of the Kharif season (June–October), the total damage in 2007 was estimated to range from US\$400,000 to 500,000 in north India alone. Apart from the yield losses, the pest infestation increased the cost of insecticide application by US\$250-375 per acre in both India and Pakistan. In a survey conducted (Nagrare et al. 2009) over 47 locations in nine cotton-growing states of India showed that two mealybug species, the solenopsis mealybug, Phenacoccus solenopsis Tinsley, and the pink hibiscus mealybug, Maconellicoccus hirsutus (Green), were found to infest cotton plants. However, P. solenopsis was found to be the predominant mealybug species, comprising 95% of the samples examined. P. solenopsis, which was hitherto not reported to occur in India, now appears to be widespread on cotton in almost all cotton-growing states of the country. P. solenopsis is an exotic species that originated from USA and was reported to damage cotton and crops of 14 families. In due course of time the species was also reported on different crops of food, fibre, fruit, ornamental, plantation, vegetable and weeds. P. solenopsis has been the topic of research for insect taxonomists and applied entomologists in India and elsewhere in the world due to its invasiveness, rapid spread, morphological and biological variations and the need for establishing an effective management strategy. This compendium presents the exclusive studies carried out on P. solenopsis besides other cotton mealybugs observed to facilitate the availability of basic comprehensive information for the researchers. It would be useful in understanding pest behaviour and devising ecofriendly management strategies based on the situation to save the cotton and other important crops infested by mealybugs.

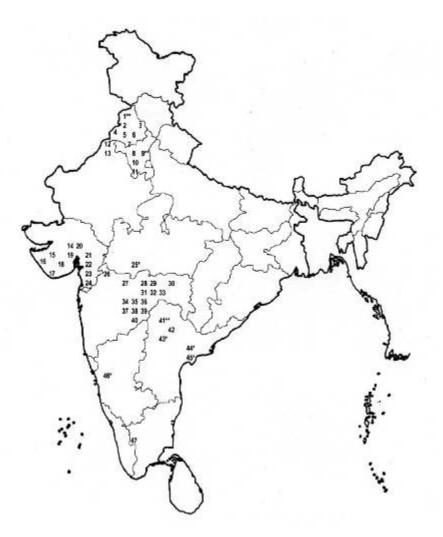
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# 2. STATUS OF MEALYBUGS ON COTTON IN INDIA

- Four mealybug species viz., the solenopsis mealybug, *Phenacoccus solenopsis* Tinsley, the pink hibiscus mealybug, *Maconellicoccus hirsutus* (Green), the Papaya mealybug, *Paracoccus marginatus* Williams and Ganara de willink and the spherical mealybug, *Nipaecoccus viridis* Newstead were found to infest cotton plants in India.
- During 2007, P. solenopsis was found to be the predominant mealybug species, comprising 95% of the samples examined from 47 locations representing 9 cotton growing states of India.
- Occurrence of *M. hirsutus* on cotton still remains rare and sporadic like it had always been in India and thus deserves least attention.
- P. solenopsis was hitherto not reported to occur in India, observed to be widespread on cotton in almost all cotton-growing states of the country. It is considered to be an exotic species that has its origin in the USA.
- P. marginatus also infests cotton and was found to be a sporadic cum potential pest in South Zone.
- The occurrence of *N. viridis* Newstead was found to be sketchy and less frequent on cotton or any other plants including weeds.
- In cotton agro-ecosystem four more species viz, Coccidohystrix insolita Green, Ferrisia virgata Cockrell, Drosicha mangiferae Green and Ferrisia malvastra (Mc Daniel) on pigeonpea, guava, mango and a weed host Sonchus oleraceus, also observed although, rare in occurrence.
- Besides all four species of cotton, *P. solenopsis* was found to infest other cultivated crops like okra, tomato, chilli, brinjal, potato, cluster bean, green gram, papaya, sunflower.
- A record of 166 host plants of *P. solenopsis* belonging to 51 families comprising 78 weeds, 27 ornamentals, 19 trees, 17 vegetables, 12 field crops, 8 fruit plants and 5 spice plants was made in three cotton agro-ecosystems of India.
- A great biodiversity of natural enemies of mealybugs have been recorded which are helping in keeping population of mealybug under check. Among them, *Aenasius bambawalei* Hayat observed to be potential parasitoid. Its presence was seen all over the country.
- Synonyms of *P. solenopsis: P. gossypiphilous* Abbas, *P. solani* Ferris, *P. defectus* Ferris, *P. gossipy.*

COMPENDIUM OF COTTON MEALYBUGS

#### 3. DISTRIBUTION OF *P. SOLENOPSIS* AND *M. HIRSUTUS* INFESTING COTTON DURING 2007



(\* M. hirsutus, \*\* Both P. solenopsis and M. hirsutus while remaining numbers showing P. solenopsis)

Punjab: Firozpur (1), Faridkot (2), Ludhiana (3), Muktsar (4), Abohar (5), Bathinda (6), Mansa (7), Haryana: Fatehabad (8), Sirsa (9), Hisar (10), Bhiwani (11), Rajasthan: Sriganganagar (12), Hanumangarh (13), Gujrat: Surendranagar (14), Rajkot (15), Porbandar (16), Junagarh (17), Amreli (18), Bhavnagar (19), Ahmedabad (20), Anand (21), Vadodara (22), Bharuch (23), Surat (24), Madhya Pradesh: Khandawa (25), Maharashtra: Dhule (26), Buldhana (27), Akola (28), Amaravati (29), Nagpur (30), Washim (31), Yavatmal (32), Wardha (33), Aurangabad (34), Jalna (35), Hingoli (36), Beed (37), Parbhani (38), Nanded (39), Latur (40), Andhra Pradesh: Karimnagar (41), Warangal (42), Hyderabad (43), Amravathi (44), Guntur (45), Karnataka: Dharwad (46), Tamil Nadu: Coimbatore (47).

# 4. NATURE OF DAMAGE BY MEALYBUGS

Mealybugs are small sap-sucking insects cause severe economic damage to cotton and a wide range of vegetable, horticultural and other field crops. Plants infested by mealybugs during vegetative phase exhibit symptoms of distorted, bushy shoots, crinkled and/or twisted bunchy leaves and stunted plants that dry completely in severe cases. Late season infestations during reproductive crop stage result in reduced plant vigour and early crop senescence. While feeding mealybugs injects a toxic substance into the plant parts resulting in chlorosis, stunting, deformation and death of plants.



1. *P. solenopsis* infested seedling, 2. Stunted growth and reduced internodal distance, 3. Reduction in boll numbers, 4. Infestation at harvesting stage

Mealybugs attacks cotton growing parts viz., main stem, branches and fruit, underdeveloped flowers produced bolls of smaller size; boll opening adversely affected resulting in serious reduction in yield. Excretion of honeydew attracts ants and also contributes to the development of black sooty mould. Plants severely affected with sooty mould have the appearance of burn symptoms. Infested cotton plant shows the symptoms like white fluffy mass on underside of leaves, near growing tips, along leaf veins and on stem, distorted or bushy shoots.

# **5. SPREAD OF MEALYBUGS**

Exotic pests, especially those that are polyphagous with a wide host range, establish themselves easily in the introduced countries, in the absence of their native natural enemies. Such insects have immense potential to emerge as crop pests, cause severe economic damage to a wide range of crops and pose a grave threat to agriculture in the introduced country. *P. solenopsis* is a polyphagous pest, with a wide host range. It establishes and spreads more easily than any other insect species because of the following factors.

- Mealybugs possess a waxy coating on the dorsal side that protects them from insecticides and natural mortality factors
- Have a high reproductive rate
- · Have the ability to hide in cracks and crevices in soil and corners of plant parts



Spread of mealybugs through 1. Plant parts, 2. Human being, 3. Farm animals and 4. Ants

- Mealybugs have the propensity to spread through natural carriers such as raw cotton, linted cotton seeds, wind, water, rain, birds, human beings, ants and farm animals.
- Being polyphagous in nature and adaptive in various climatic conditions, mealybugs poses tremendous threat to agricultural crops worldwide.

# 6. GLOBAL DISTRIBUTION AND PEST STATUS OF P. SOLENOPSIS

Year	Region /country	Reported by	Host plants
1898	New Mexico, USA	Tinsley (1898)	Weed roots in a nest of the ant, <i>Solenopsis geminata</i> Fab.
1966	Hawaii	Kumashiro et al. (2001)	
1990	Texas, USA	Fuchs et al. (1991)	Cotton and other hosts
1992	Central America	Williams and Granara de Willink (1992)	
1994	Caribbean and Ecuador	Ben-Dov (1994)	-
2002	Chile	Larrain (2002)	<i>Solanum muricatum</i> (Ait) (Fam.: Solanaceae)
2003	Argentina	Granara de Willink (2003)	False ragwood, <i>Ambrosia tenuifolia</i> Spreg. (Fam.: Asteraceae)
2005	Brazil	Culik and Gullan (2005)	Tomato, <i>Solanum</i> <i>lycopersicum</i> L.(Fam.: Solanaceae)
2005-09	Pakistan	Abbas <i>et al.</i> (2005) Zaka <i>et al.</i> (2006) Muhammad (2007) Arif et al. (2002)	Cotton
		Arif et al. (2009)	154 host plants including Cotton
2006	Thailand and Taiwan	Abbas <i>et al</i> . (2010) Hodgson <i>et al</i> . (2008)	Cotton
2006-09	India	Dhara Jothi <i>et al.</i> (2008) Jhala <i>et al.</i> (2008) Jhala and Bharpoda (2008) Nagrare <i>et al.</i> (2009) Monga <i>et al.</i> (2009)	Cotton
2008	Nigeria	Akintola and Ande (2008)	Hibiscus rosa-sinensis
2009	Sri Lanka	Prishanthini and Laxmi (2009)	Ornamentals, vegetable crops and weeds
2009	China	Wang <i>et al.</i> (2009) Wu and Zhang (2009) Wang <i>et al.</i> (2010)	Cotton
2009	USA	Ben-Dov et al. (2009)	183 host plants including cotton
2010	Australia	Admin (2010), Robinson and Tapim (2010)	Cotton

# 7. COTTON INFESTED BY DIFFERENT SPECIES OF MEALYBUGS

#### Phenacoccus solenopsis Tinsley

An introduced mealybug species *P. solenopsis* has been causing serious damage to cotton in India since 2006. The outbreaks have been serious on both Bt and non-Bt cotton, and growers have resorted to applying large amounts of pesticides at frequent intervals. *P. solenopsis* was mainly found on the young growth, including twigs, leaves, flower buds and petioles, but can occur even on the stems in heavy infestations. The infested plants become stunted, and most plants look dehydrated. In severe outbreaks, the bolls fail to open and defoliation occurs. In addition, the plants become covered in a dense mat of sooty moulds that grow on the large amount of exuded honeydew. This honeydew also attracts ants, which probably defend the mealybugs from attack by predators and parasitoid wasps.



1. Full grown female 2. Ovisac, 3. Colonies 4. Infested plant

Body oval, often quite large (5 mm); somewhat rounded in lateral view; yellowish–green; legs red; covered by thin, white, mealy wax, with dark dorso-submedial bare spots on intersegmental areas of thorax and abdomen, these areas forming 1 pair of dark longitudinal lines on dorsum; with 18 pairs of lateral wax filaments, posterior pairs longest, up to  $1/4^{th}$  of length of the body

#### Paracoccus marginatus Williams and Granara de Willink

Papaya mealybug *P. marginatus* was recorded in a severe form for the first time on cotton in Coimbatore. Infestation of *P. marginatus* was observed on *G. arboreum* and *G. hirsutum* species including Bt - cotton under field conditions. It was observed that severe infestation lead to stunted growth and drying of the sympodial branches. The mealybug infestation was observed as clusters of cotton-like masses on the leaf, squares and bolls. Mealybug caused havoc in agricultural and horticultural crops ever since its first report from Coimbatore in 2007. The insect assumed the status of a major pest in 2009 when it caused severe damage to economically important crops and huge losses to farmers in Coimbatore, Erode, Tirupur and Salem districts of Tamil Nadu (Tanwar *et al.* 2010).

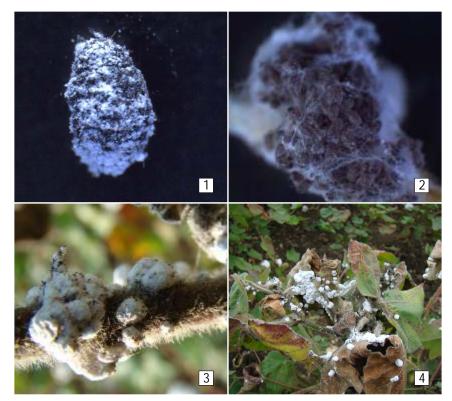


1. Full grown female 2. Ovisac, 3. Colonies, 4. Infested plants

The adult female is yellow, covered with a white waxy coating and measures approximately 2.2 mm long and 1.4 mm wide. A series of short waxy caudal filaments less than one-fourth the length of the body exist around the margin. Eggs are greenish yellow and are laid in an ovisac that is three to four times the body length and entirely covered with white wax. The ovisac develops on the underside of an adult female.

#### Nipaecoccus viridis (Newstead)

*N. viridis* is an agricultural pest in Asia that attacks food, forage, ornamental crops and fiber crops, such as cotton. It is also a pest of stored potatoes. *N. viridis* is widespread throughout the tropics and subtropics, attacking numerous plant species and often causing considerable damage. The potential for invasiveness appears high. Cotton plants infested by mealybugs exhibit symptoms of distorted and bushy shoots, crinkled and/or twisted and bunchy leaves, and stunted plants that dry completely in severe cases. Late season infestations result in reduced plant vigour and significant reduction in yield.



1. Full grown female 2. Ovisac 3. Colonies 4. Infested plants

Body round or broadly oval; somewhat flattened dorsoventrally; purple or blue green; covered by thick white, creamy, or pale yellow wax, without bare areas on dorsum; ovisac covering dorsum; probably with 5 or 6 pairs of lateral wax filaments. Approximately 4 mm long by 3 mm. Females produce an ovisac with a wax that is sticky when touched. In high densities, waxy secretions may appear as a continuous layer of wax thereby preventing the detection of individual mealybugs.

#### COMPENDIUM OF COTTON MEALYBUGS

# 8. ASSESSMENT OF MEALYBUG INCIDENCE AND SEVERITY

The incidence and severity based on presence of mealybugs can be assessed using one to four scales of infestation viz.,

- I Scattered appearance of few mealybugs on the plant;
- II Severe infestation of mealybug on any one branch of the plant;
- III Severe infestation of mealybug on more than one branch or half portion of the plant;
- IV Severe infestation of mealybug on the whole plant.



1. Grade I, 2. Grade II, 3. Grade III, 4. Grade IV

Per cent incidence (PI)= Number of infested plants / total plants observed x 100 Severity Index (SI)= Sum of total grade points (1-4 for infestation grade G-I to G-IV, respectively) of infested plants / Total number of infested plants observed

#### Sampling of mealybug infestation

Study on sample size indicated the importance of locating the source of mealybug infestation first, and sampling that accounts field areas largely parallel to the infestation source. While sample sizes of 25 to 50 plants per acre are sufficient in fields with known source of infestation such as roadside weeds and water channels, 100 plants per acre have to be sampled in clean field where prior knowledge of mealybug infestation is not available.

#### 9. FIELD DYNAMICS AND INTENSITY OF P. SOLENOPSIS AND P. MARGINATUS

Cotton- wheat cropping system (North zone )

*P. solenopsis* population was seen during the entire crop season with high population at 26 Standard Week (SW) with intensity 1.17, gradually declined over the season. At 45 SW, infestation was 39 % with corresponding severity index 2.79 (Fig 1). The reduction in yield of cotton plants was estimated to be 14.87 and 51.86 % for Grade I and Grade IV mealybug infestation levels, respectively during 2009 in comparison to 16.63 and 53.65 % for grade I and grade IV, respectively during 2008.

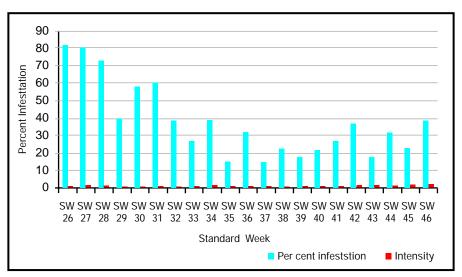


Fig 1. Per cent infestation of *P. solenopsis* during the crop season

Cotton + Pigeon pea – Fallow cropping system (Central zone)

Mealybug infestation in cotton + pigeon pea cropping system of central zone was in traces and sporadic during 2008-09 crop season at research station and farmer fields. However, surveys revealed their occurrence at random across locations on diverse hosts.

Cotton + Pulse - Maize cropping system (South zone)

Population dynamics of *P. marginatus* and *P. solenopsis* were observed under cotton+cowpea intercropping system at the experimental station during 09-10. *P. marginatus* dominated over *P. solenopsis*. *P. solenopsis* had lower per cent incidence (PI) ranging from 17 to 43% as well as severity index (SI) from 0.20 to 0.43. The PI & SI was higher in *P. marginatus*, it ranges from 36 to 96 (Fig 2) and 1.10 to 2.37 (Fig 3), respectively.

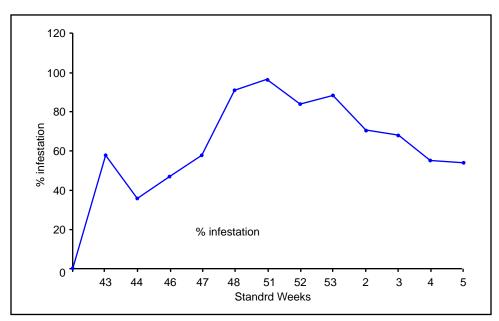


Fig 2 : Population dynamics of *P. marginatus* (09-10) in South zone

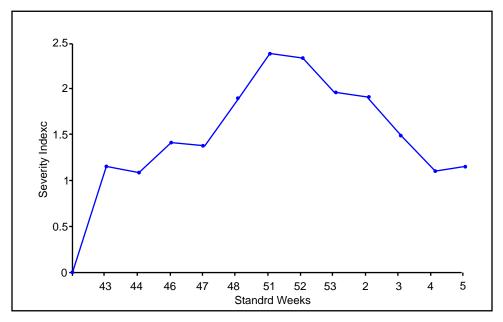


Fig 3 : Severity of *P. marginatus* (09-10) in South zone

# **10. GROWTH PARAMETERS OF MEALYBUG ON COTTON**

Growth parameters of cotton mealybugs *P. marginatus* and *P. solenopsis* were observed under laboratory condition on host cotton (Table 1). The mortality rate was high during first 10 days for *P. solenopsis* and 6 days for *P. marginatus*. *P. solenopsis* adults started laying eggs after 28 days and ceased after 32<sup>nd</sup> day. *P. marginatus* started laying eggs after 24 days and ceased after 28<sup>th</sup> day. The capacity for increase was slightly less than intrinsic rate of increase indicating that the population was tending towards overlapping generation.

Table 1: Population growth parameters of mealybugs on cotton

Parameter	<i>P. marginatus</i> n = 500	P. solenopsis n = 500
Gross reproduction rate (GRR)	497	532
Net reproductive rate (Ro)	176.08	157.17
Mean length of generation (Tc)	26.58	30.36
Innate capacity for natural increase (r.)	0.1945	0.1665
True intrinsic rate of increase $(r_m)$	0.1952	0.1760
True generation time (T)	26.49	28.73
Finite rate of increase (?)	1.2155	1.1924
Doubling time (DT)	3.55	3.94
Annual rate of increase	9.09 x 10 <sup>30</sup>	7.93 x 10 <sup>27</sup>

Where, GRR-Total number of eggs laid per female

Ro-Number of females produced in each generation

r<sub>c</sub>- Capacity of species to increase in number (approximate)

 $r_m$ - Capacity to increase in number (accurate)

Finite rate of increase ()-Number of times a population increase per unit time

DT- Time taken by species to double its population

#### COMPENDIUM OF COTTON

MEALYBUGS

# 11. TAXONOMIC IDENTIFICATION OF PHENACOCCUS SOLENOPSIS TINSLEY

Family : Pseudococcidae

- Elongate to broadly oval shaped body (ps)
- Antennae with 6-9 segments (ps-a)
- Three segmented labium with apicoventral setae on each side of the terminal segment (ps -b)
- Legs well developed; claw with a pair of digitule and with or without denticle, tarsal digitules setose or knobbed; translucent pores present at least on the tibia (ps -c,d,e)
- Tubular ducts present (ps -f)
- Presence of anterior and posterior pairs of ostiole (ps-g)
- Anal ring at the apex with 2 rows of cells and 6 slender setae (ps-h)
- Presence of trilocular pores on the dorsum and venter (ps-i)
- Cerarii with conical setae accompanied by a concentration of trilocular pores, and situated on the margins (ps-j)
- Presence of circulus between III and IV abdominal segments (ps-k)
- Presence of multilocular disc pores (ps-I-s)

Sub-family: Phenacoccinae

- Nine segmented antennae (ps-a)
- Tarsal digitules setose (ps-d); claw with denticle (ps-d).

#### Genus : Phenacoccus

- Broadly oval body (ps)
- Nine segmented antennae (ps-a),
- Well developed legs (ps-c) with denticle on claw (ps-d) and tarsal digitules flagellate (ps-d).
- Translucent pores on tibia and femur (ps-e)
- Oral collar tubular ducts on venter (ps-f)
- Cerarii numbering 18 pairs (ps), each cerarii with a pair of conical setae and trilocular pores (ps-j)
- Circulus present (ps-k)

Species : solenopsis

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CICR

- Nine segmented antennae (ps-a)
- Translucent pores on the apex of the hind femur and tibia (ps-e)
- Large and flaccid circulus (ps-k)
- Multilocular disc pores more concentrated near the region of vulva or restricted in the segments VI to VIII (ps-q,r,s) (Fig. 4)

[Source: Dr V. V. Ramamurthy, IARI, New Delhi]

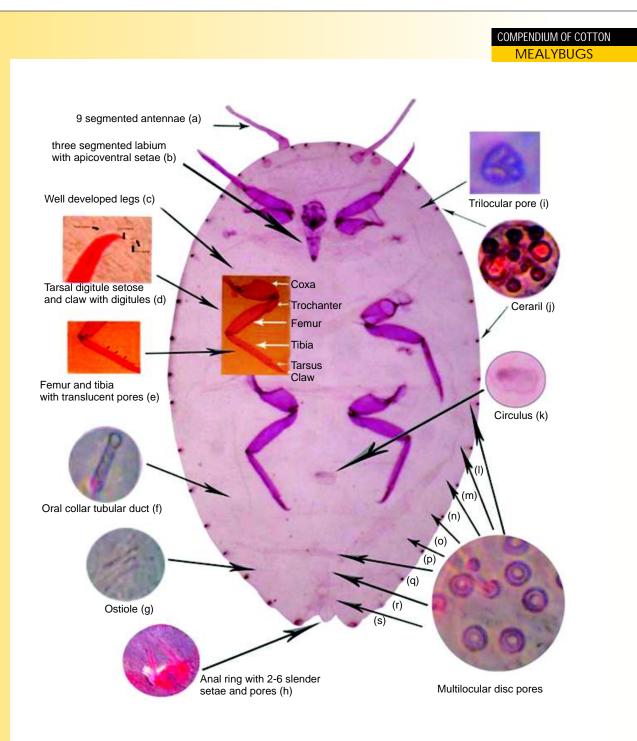


Fig 4. *Phenacoccus solenopsis* Tinsley (Female) [Source: Dr V. V. Ramamurthy, IARI, New Delhi]

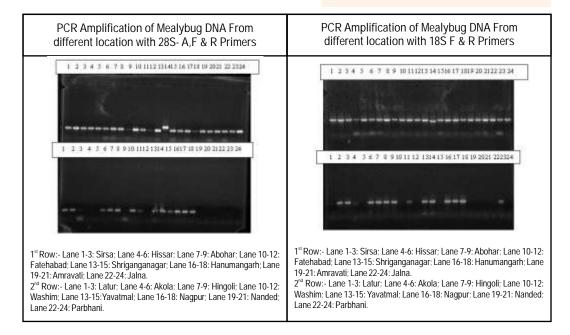
# 12. MOLECULAR CHARACTERIZATION OF P. SOLENOPSIS

Live mealybug colonies of *P. solenopsis* were collected in October- November 2007. DNA was isolated from 700 healthy female mealybugs from 37 locations in the F1 generation, PCR amplified using specific primers to amplify 3 nuclear genes. Primers were designed at CICR, Nagpur and were used for amplification. PCR amplicons of approximately 350 bp and 700 bp were sequenced using double pass analysis in 140 samples representing 3-4 samples per location. All of them had the identical sequence, with minimal genetic diversity (molecular analysis) throughout the country

COMPENDIUM OF COTTON MEALYBUGS

18S - A	- F CTGGTTGATCCTGCCAGTAG
18S - A	- R CCGCGGCTGCTGGCACCAGA
28S - A	- F AGAGAGAGTTCAAGAGTACGTG
28S - A	- R TTGGTCCGTGTTTCAAGACGGG
28S - E	3 - F GTAGCCAAATGCCTCGTC
28S - E	3 - R CACAATGATAGGAAGAGCC
EF-1	- F CACATYAACATTGTCGTSATYGG
EF-1	- R CTTGATGAAATCYCTGTGTCC
EF-1	- B-FCARGACGTATACAAAATCGG
EF-1	- B-RGCAATGTGRGCIGTGTGGCA

Primers used for amplification of 18S, 28S rDNA genome.



Five Mitochondrial DNA of *P. solenopsis* characterized and submitted to gene bank with following accession numbers.

ACCESSION FJ526385 ACCESSION FJ526384 ACCESSION FJ526383 ACCESSION FJ526382 ACCESSION FJ526381

[Source: Technology Mission on Cotton - Mini Mission I 3.1: Emerging and key pest]

## 13. PROTOCOL FOR STUDIES ON BIOLOGY OF *P. SOLENOPSIS* UNDER LAB CONDITIONS

Protocol for the studies on the biology of *P. solenopsis* in the laboratory on host cotton was standardized at CICR, Nagpur. Individual fresh cotton leaves with petioles of the cotton plant (*G. hirsutum*) were collected. Washed with tap water and dried. Base of the petiole of individual leaves with a water soaked cotton swab was covered to prevent desiccation of the leaf. Individual neonate crawlers emerged from females were used to start the biology study. Crawlers drawn from different females but laid on the same day individually were transfered to separate glass Petri plates (15 X 2 cm) each containing a cotton leaf. Observations on survival and molt of the crawlers were recorded daily under stereoscopic microscope until they became adults. Unless the crawlers were in a pre-molt stage, petri plates along with cotton leaves were changed on alternate days or else they were transferred after the molt.



1. Washing of leaves, 2. Drying, 3. Covering base of the petiole with damp swab, 4. Release of mealybug on cotton leaf in Petri plate

Petri plates with missing crawlers were discarded and excluded from the final data. Developmental time of each instar based on observed exuvia was recorded. Crawlers were monitoring every day and those leaf dries harbouring eggs were separated and were observed until hatching. The crawlers that had stopped further moulting and reached adult stage were used to determine the prereproductive, reproductive periods, fecundity, and longevity. After counting the eggs or neonate crawlers, individual adults were transferred to new petri plates for further observations.

# 14. BIOLOGY OF P. SOLENOPSIS (FEMALE)

At mean temperature and relative humidity of  $23.3-30.2^{\circ}$ C and 40.5-92.5% RH, the developmental period from immature crawler to adult stage was  $13.2 \pm 1.8$  days. Survival of second instars was lower (45.5%) than first and third instars (71.4%). Females showed dynamic patterns of fecundity with the number of crawlers produced per female ranging between 128 and 812, with a mean of  $344 \pm 82$ . The reproductive period lasted  $30.2 \pm 8.2$  days. Parthenogenesis with ovoviviparity (96.5%) was dominant over the oviparous (3.5%) mode of reproduction. Adult females lived  $42.4 \pm 5.7$  days.



Female life stages 1. Egg, 2. First instar, 3. Second instar 4. Third instar

Offspring production by females was disjunctive with one to seven non-reproductive periods with a mean of  $2.4 \pm 0.6$  days interspersed between the reproductive phases of the life cycle. More than 10 crawlers per day were produced by females for a minimum and maximum period of 6 and 30 days,, with a mean effective reproductive period of 17.2  $\pm$  4.3 days, during which 97.3% of crawlers were produced. (Vennila *et al.*, 2010)

# 15. BIOLOGY OF P. SOLENOPSIS (MALE)

The developmental period of crawlers was shorter and similar for first and third instars (2-6 days), and longer for the second instar (2-11 days). Mean developmental periods of first, second and third instar were  $3.9 \pm 0.4$ ,  $5.1 \pm 3.2$  and  $4.2 \pm 0.6$ , respectively. Males had an additional instar and prepupal stage over 5-7 days of development with a mean of  $5.5 \pm 0.5$  days. Males were winged, delicate and non-feeding. The proportion of males to the total population used in the study was 5%, and they lived for a maximum of 2 days with a mean of  $1.5 \pm 0.1$  days.



Male life stage1. Third moult, 2. Fourth moult, 3. Adult ready for emergence 4. Adult male

Males are quiet small, total-body length about 1.41 mm; antennae about 2/3rds total-body length; body with few setae, all hair-like and fleshy; present on antennae and legs; length of antennae a little less than twice width of antennal segment; loculate pores mainly with 4, but occasionally 5, loculi. Abdomen with glandular pouches and glandular pouch setae on segments VII and VIII. Penial sheath with a distinct constriction towards apex. Wings misshapen and length unknown (Hodgson *et al.* 2008). Two pairs of terminal filaments present.

# 16. LAB REARING AND MULTIPLICATION OF P. SOLENOPSIS

*P. solenopsis* can easily be reared and multiplied under laboratory conditions at the temperature range between 15-30 °C and relative humidity 50-90%. Fine field soil mixed with vermicompost (1:1) was filled (about 1-1.5 inch) in a plastic container (12 inch dia.), 6-10 sprouted potato were placed (depending upon size of potato) on the soil. The soil was moistened; culture of *P. solenopsis* was released and allowed to settle. Mealybug population was ready in a month from release of culture.



Media preparation for growing potato, 2. Potato placed on soil,
One month after mealybug population, 4. Watch on mealybug multiplication

#### Precautions

- 1. Do not underwater or overwater potato growing media.
- 2. Remove rotton potato, as and when noticed and dispose it off.
- 3. Collect the mealybugs by camel brush that are settled at the corners of the container or parts other than the plants and shift them on plant parts every day.
- 4. While working care should be taken that mealybugs are not transferred outside culture room on the clothes of lab workers.
- 5. Always change the potato growing media at each fresh culturing of mealybugs.
- 6. Use disease free/undamaged potatoes.

#### COMPENDIUM OF COTTON MEALYBUGS

# 17. LAB BIOASSAY AGAINST P. SOLENOPSIS

For conducting lab bioassay against mealybug *P. solenopsis* following protocol can be adopted to assess the efficacy of insecticides.

- 1. Prepare half litre insecticidal test solution in a one litre container at desired dose.
- 2. Dip 9 medium size fresh sprouted potatoes in insecticide solution for 5 minutes.
- 3. Remove potatoes from test solutions and keep for drying on plain paper for 2 hours inside room under fan.
- 4. Place three potatoes each on moist soil in plastic container (size 5" dia. x 6" ht.) in three replications.
- 5. Release fifty 3<sup>rd</sup> 4<sup>th</sup> instar nymph of *P. solenopsis* in each container.
- 6. Secure the mouth of container with perforated lid or muslin cloth.
- 7. Keep container at room temperature max.  $27\pm2^{\circ}$ C and min.  $17\pm2^{\circ}$ C and RH about  $60\pm5\%$



1. Drying of treated potatoes 2. Lab cultured mealybugs to be used for bioassay,

- 3. Collection of mealybugs of desired size and number, 4. Containers with treatments
- 8. Record dead mealybugs by taking out all the mealybugs on black paper at 4 and 8 days after treatment.
- 9. Correct per cent mortality by using Abbot's (1925) formula.

# 18. BEHAVIOUR OF *P. SOLENOPSIS* ON SOME GROWTH PARAMETERS

Lab experiments were carried out to study the developmental rates of *P. solenopsis* at different constant temperatures viz. 25, 27, 30 and  $32^{\circ}$ C. The mean fecundity was maximum 434.4 ± 155.21 (range 281-723) at 25°C and found to be decreasing with increase in temperature. The number of eggs observed per female also showed increasing trend. However the incubation period varied and did not demonstrate any trend, this may be due to hatching of single egg laid by female. Hatching takes duration of few minutes to an hour. Nymphal developmental period was maximum at 25°C in all instars (Total duration in range of 22 to 44 days) and found to be less in subsequent temperatures except 32°C where nymphal period increased by few days (Total duration in range of 18 to 34 days), increased with decrease in temperature (Fig 5-8).

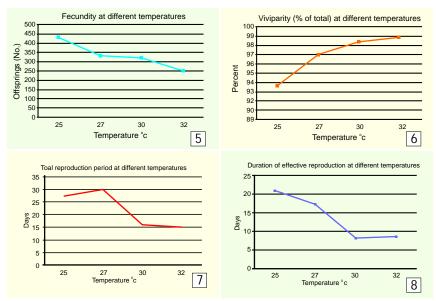


Fig 5. Fecundity of *P. solenopsis*, Fig 6. Per cent viviparity, Fig 7. Total reproduction period, Fig 8. Duration of effective reproduction

Growth parameters	25 °C		27 ° C		30 ° C		32 ° C	
	Mean± SD	Range	Mean	Range	Mean± SD	Range	Mean± SD	Range
Fecundity (No.)	434.4 ± 155.21	281-723	334.41	128-812	319.38 ± 110.5	182 - 552	253.6 ± 183.4	112 - 713
Viviparity (% of total)	92.9		96.54	92-100	98.23 ± 1	96.49 - 99.72	98.71	
Total reproduction period (Days)	27.33 ± 3.67	21 - 32	30.23	10-47	15.93 ± 6.10	6 - 28	14.8 ± 4.24	11 - 21
Duration of effective reproduction (Days)	20.9 ± 5.4	13 - 27	17.16	6-30	8.37 ± 3.82	4 - 17	8.5 ± 2.22	5 - 13

Adult female longevity was maximum at both 25 and 27°C temperatures. Duration of effective reproduction days was seen to be decreasing in trend with increase in temperature. The percentage of offsprings produced by female during the period of effective reproduction were 99.72% at 25°C and showed reducing rate at increased temperature.

COMPENDIUM OF COTTON MEALYBUGS

# 19. HOST PLANTS OF P. SOLENOPSIS

A record of 166 host plants belonging to 51 families comprising 78 weeds, 27 ornamentals, 18 trees, 17 vegetables, 12 field crops, 8 fruit plants and 4 spice plants was made in three cotton agro-ecosystems of India. Maximum host plants were recorded from Malvaceae (10.24%) followed by Asteraceae (9.64%), Fabaceae (9.04%), Amaranthaceae (6.63%), Euphorbiaceae (6.63%), Solanaceae (6.02), Poaceae (4.22%) and Lamiaceae (3.61%). Out of 39 G-IV hosts, 11 were from Malvaceae followed by 7 of Asteraceae and 5 Solanaceae. Three G-IV hosts each were recorded from Fabaceae and Euphorbiaceae and 2 from Tiliaceae.



1. Tomato 2. Congress Grass 3. China Rose 4. Roselle

A single G-IV host was also recorded from Aizoaceae, Acantheceae, Amaranthaceae, Caricaceae, Nyctaginaceae, Portulacaceae, Rutaceae and Verbenaceae. Of the 47 cultivated crops recorded, cotton, okra (Malvaceae), tomato, chilli, brinjal, potato (Solenaceae), cluster bean, green gram (Fabaceae), papaya (Caricaceae), sunflower (Asteraceae) had severe infestation of mealybug. Host plants with G-IV infestation- *T. portulacastrum* Linn. (Aizoaceae); *P. hysterophorus* Linn. (Asteraceae); *C. papaya* Linn. (Caricaceae); *A. indicum* Linn., *H. rosa-sinensis* Linn., *A. esculentus* (Malvaceae); *L. esculentum* Mill., *P. minima* (Solanaceae) were the most preferred hosts in all the three cotton agro-ecosystems (Table 2).

# COMPENDIUM OF COTTON MEALYBUGS

SI No	Family/ Botanical name	Common name	Host type	Cotton agro- ecosystems 1, 2, 3 <sup>s</sup>	Growing season <sup>##</sup>	Growth stage*	Location of the host**
	Aizoaceae						
1.	Trianthema.	Giant pigweed	Weed	1, 2, 3	S, Os	v, f, m	F, B, R W
	portulacastrum Linn						
	Acantheceae						
2.	Asteracantha longifolia Nees	Water spiny ball	Weed	2	S	v, f, m	F, B, R
	Amaranthaceae						
3.	Digera muricata (Linn.)	False amaranth	Weed	2	S	v, f, m	F, B
	Asteraceae						
4.	Acmella uliginosa (SW.) Cass.	Marsh para cress	Weed	2	S, Os	v, f	F,B
5.	Helianthus debilis Linn.	Beach sunflower	Ornamental	1	S	f, m	F
6.	Helianthus sp.	Wild sunflower	Weed	1	S, Os	v, f, m	F, R, W
7.	Parthenium hysterophorus Linn.	Congress grass	Weed	1, 2, 3	S, Os	v, f, m	F,B,R
8.	Pentanema indicum (L.) Y. Ling	Sonkadi	Weed	2	Os	f, m	F,B
9.	Taraxacum officinale Linn.	Dandelion	Weed	2	Os	v, f	R
10.	Xanthium strumarium Linn.	Common cocklebur	Weed	1,2	S	v, f, m	F, R, W
	Caricaceae						
11.	Carica papaya Linn.	Papaya	Fruit plant	1, 2, 3	S, OS	v, f, m	F
	Euphorbiaceae						
12.	Euphorbia heterophylla Linn	Wild Poinsettia	Weed	2, 3	S, Os	v, f, m	F,B,R,W
13.	Euphorbia hirta Linn.	Common spurge	Weed	2,3	Os	v, f, m	F,B,R,W
14.	Phyllanthus amarus Linn.	Niruri	Weed	3	S,Os	v, f	F,B,R
	Fabaceae						
15.	Clitoria ternatea Linn.	Butterfly Pea	Weed	2	Os	v, f, m	F, R
16.	Cyamopsis tetragonoloba(Linn.)	Cluster bean	Vegetable	1	S	v, f, m	F
17.	Vigna radiata Linn.	Greengram	Field crop	1	S	v, f, m	F
	Malvaceae						
18.	Abelmoschus esculentus Linn.	Okra, Lady's finger	Vegetable	1, 2	Os	v, f , m	F
19.	Abelmoschus ficulneus (Linn.)	Native rosella	Weed	2	S, Os	f, m	F, B
20.	Abutilon indicum Sweet	Indian mallow	Weed	2,3	S, Os	v, f, m	F, B, R,W
21.	Gossypium arborium Linn.	Indian cotton	Field crop	1, 2, 3	S	v, f, m	F
22.	Gossypium barbadense Linn.	Egyptian cotton	Field crop	3	S	v, f, m	F
23.	Gossypium herbaceum Linn.	Mexican cotton	Field crop	1,2	S	v, f, m	F

# Table 2: Severely infested host plants (Grade IV) of P. solenopsis in India

#### COMPENDIUM OF COTTON MEALYBUGS

24.	Gossypium hirsutum Linn.	American cotton	Field crop	1, 2, 3	S	v, f, m	F
25.	Hibiscus rosa-sinensis Linn.	China rose	Ornamental	1, 2, 3	S, Os	-	R
26.	Hibiscus sabdariffa Linn.	Roselle	Vegetable	2	S, Os	v, f, m	F
27.	Malvastrum coramandelinum G.	False mallow	Weed	2	S, Os	v, f, m	F,B
28.	Sida cordifolia Linn.	Heart-leaf sida	Weed	1	S, Os	v, f , m	R
	Nyctaginaceae						
29.	Boerhavia diffusa Chois.	Red hogweed	Weed	2	S, Os	v, f	F,B, R
	Portulacaceae						
30.	Portulaca oleracea Linn.	Purslane	Weed	3	S	f, m	B, R, W
	Rutaceae						
31.	Murrya koenigii Spreng	Curry leaf	Spice	2,3	Os, S	-	B, R
	Solanaceae						
32.	Capsicum annum Linn.	Chilly	Spice	2	S	v, f, m	F
33.	Lycopercicon esculentum Mill.	Tomato	Vegetable	1, 2, 3	S, Os	v, f, m	F
34.	Solanum melongena Linn.	Brinjal	Vegetable	1,2	S, Os	v, f, m	F
35.	Solanum tuberosum Linn.	Potato	Vegetable	2,3	S	v, f, m	F
36.	Withania somnifera (L.) Dunal.	Winter cherry	Ornamental	1	S, Os	v, f, m	R, W
	Tiliaceae						
37.	Corchorus trilocularis Linn.	Wild jute	Weed	2, 3	S, Os	v, f, m	F,B,R
38.	Triumfetta rhomboidea Linn.	Chinese burr	Weed	2	S, Os	v, f, m	F,B,R
	Verbenaceae						
39.	Lantana camara Linn.	Lantana	Ornamental	2,3	Os	-	B,R,W

<sup>1</sup> Irrigated cotton agro-ecosystem of North India, <sup>2</sup> Rainfed cotton agro- ecosystem of Centra India and <sup>3</sup> Partially irrigated cotton agro-ecosystem of South India	al
S = Cotton season. Os = Off-season	
v = vegetative, f = flowering and m = maturity	
F = field, B = field boundary, R = road side, W = near water source	
	S = Cotton season. Os = Off-season

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#### 20. DIVERSITY OF BIOAGENTS OF MEALYBUGS

Bioagents from diverse groups have been recorded against different species of mealybugs across India. Among them *Aenasius bambawalei* Hayat was found to be the prominent parasitoid present all over India throughout year with parasitization potential from 5-100 percent, average 30% depending upon season and host on which mealybugs fed. Other parasitoids also recorded a parasitization ranging from 2-22 per cent. Inhabitant beetle species such as *Cheilomenes sexmaculata, Rodolia funida, Scymnus, Nephus regularis,* etc. were present in different ecosystems that feed on naturally occurring infestation. These predator and parasitoids have to be conserved and used for effective pest management so that indiscriminate use of insecticides can be avoided. In preliminary laboratory studies conducted at CICR, Nagpur, the predatory beetle, *Cryptolaemus montrouzieri* (Mulsant), which is a naturalized predator of mealybugs in India, was found to feed voraciously on *P. solenopsis*. This beetle can be released, prior to the cotton season, on weeds and perennial trees where mealybug colonies are found, and during the season on infested cotton plants. Bioagents of different mealybugs are summarized in Table 3.

Name of bioagents Parasitoid	Host mealybug	% parasitization	Distribution
Aenasius bambawalei Hayat	P. solenopsis	5-100	All over India
Metaphycus sp.	P.solenopsis	7-10	Central India
Aprostocetus bangaloricus Narendran	P. solenopsis	4-10	Central India
Encyrtus aurantii (Geoffroy)	P. solenopsis	2-9	Central India
Prochiloneurus pulchellus Silvestri*	P. solenopsis	5-10	Central India
Anagyrus dactylopii (Howard)	P. solenopsis	5-7	Central India
Anagyrus mirzai Agarwal and Alam	P. solenopsis	3-9	Central india
Homalotylus albiclavatus (Agarwal)	P. solenopsis	5-10	Central India
Anagyrus kamali Moursi	P. solenopsis	5-10	Central India
Chartocerus kerrichi (Agarwal)	P. solenopsis	6-9	Central India
Pachyneuron leucopiscida Mani	P. solenopsis	5-9	Central India
Promuscidea unfasciativentris (Girault)*	P. solenopsis/	7-22	Central India
	N. viridis		
Prochiloneurus aegypticus*	P. marginatus	2-9	South India
Predator		% Predatory potential	
Cryptolaemus montrouzieri (Mulsant)	P. solenopsis	40-50	All over India
Crysoperla carnea (Stephens)	P. solenopsis	20-40	Central India
Cheilomones sexmaculata (Fabricius)	P. solenopsis	20-30	All over India
Scymnus sp.	P. solenopsis	20-30	All over India
Rodolia funida	P. solenopsis	10-20	Central India
Nephus regularis (Sicard)	P. solenopsis	10-20	Central India
Gitonides perspicax Knab	N.viridis	33-90	Central India
Spalgis epius Westwood	P. marginatus	55-66	South India

Table 3. Diversity of bioagents of mealybugs and their distribution in India.

\* Hyperparasitoid

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# Biodiversity of mealybug parasitoids



A. bambawalei, 2. P. unfasciativentris, 3. Metaphycus sp., 4. A. kamali,
5. Torumus sp. 6. P. aegypticus, 7 & 8. Unidentified

# 

# **Biodiversity of mealybug predators**

C. montrouzieri, 2 & 3 Scymnus sp., 4. C. sexmaculata (grub), 5. Brumus sp (grub),
C. carnea (grub), 7. Spalgis epius (butterfly), 8. G. perspicax (fly)



# 21. POTENTIAL OF PARASITOID A. BAMBAWALEI

With widespread occurrence of *P. solenopsis* on cotton and diverse host plants, natural enemies also proliferated in large number in the cotton agro-ecosystem. One of the important parasitoids *A. bambawale* played a very significant role in keeping mealybug population under control. The parasitization is dependent upon the density of population. Presence of *A. bambawalei* was noticed with parasitization ranging from 5-100 per cent across the country with an average 30%. This parasitoid has some of the characteristics which can qualify as ideal parasitoid viz., 1) adaptable to the environmental condition (tolerable temperature max. 45°C, min 2°C), 2) host specific, 3) multiply faster than the host, 4) short life cycle (17-20 days) and high female:male ratio, 5) high host searching capacity, 6) amenable for easy culturing in laboratory, 7) high dispersal capacity, 8) synchronise life cycle with host.



1. *A. bambawalei* (male, female), 2. Parasitization of mealybugs on cotton plant 3. Drastically reduced mealybugs, 4. Recovery of plant

Female: Length 1.56–2.22mm. Body shiny black; head with large thimble-like setigerous punctures. Antenna with radicle black; scape testaceus yellow, with a brownish patch in middle. Fore wings infuscate in about basal third, the infuscation becoming faint distally, and hyaline in distal half and the costal cell; hind wings hyaline. Male: Length 1.04–1.45 mm, smaller than female, the sculpture and colour of mesothoracic dorsum, in antennal structure, fore wing venation and genitalia. Head colour and thimble-like punctures about same as in female. Antenna dark brown to black; scape with a white streak from basal third to apex on outer surface (Hayat, 2009).

# 22. LIFE STAGES AND LAB REARING OF A. BAMABAWALEI

*A. bambawalei* completes its life cycle inside the mealybug body. Dissection of parasitized mealybug showed presence of larva, cocoon, fully developed adult. The adult emerged out from the exit hole of cocoon. The parasitoid can directly be multiplied on the mealybugs in the lab. About 100 mealybugs with 3 pairs of *A. bambawalei* can be released in 1 plastic container (1 litre volume) with 3 sprouted potatoes and covered with muslin cloth. After 17-20 days the adults started emerging out from the mumified cocoon.

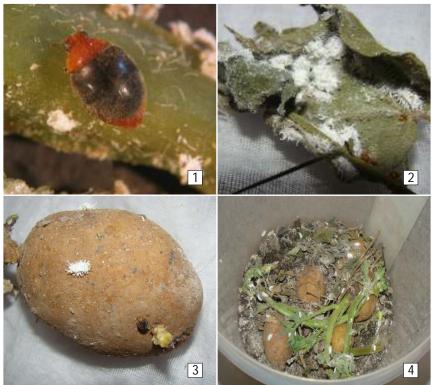


1. Larva of *A. bambawalei*, 2. Cocoon, 3. Fully developed adult, 4. Exit hole of cocoon, 5. Adult female, 6. Multiplication under lab

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## 23. MEALYBUG PREDATOR: CRYPTOLAEMUS MONTROUZIERI

*Cryptolaemus montrouzieri* (Mulsant) is a predator not only on mealybug but on several soft bodied insect pests. *C. montrouzieri* was found to feed voraciously on *P. solenopsis*. The beetle can be released on weeds and perennial trees prior to the cotton season and during the season on infested cotton plants. Both larvae and adult feed on the mealybug crawlers. The beetle can be multiplied directly on the mealybugs cultured on the sprouted potato similar to mealybug multiplication. Being a voracious predator on the host mealybugs plenty of them need to be added as feed every alternate day into the rearing container. Both the larval and adult stages of this predator attack all stages of mealybugs



1. Adult beetle 2. Congregation of grubs, 3 Grub & adult feeding on crawlers, 4. *Cryptolaemus* multiplication in plastic container

Adult beetles are dark brown with orange heads and tails, 4-5mm long. The life cycle is completed in about 31 days at 27°C and 45 days at 21°C. Eggs are laid among the cottony egg masses of mealybugs; they hatch in 5-6 days at 27°C. Females lay 5-10 eggs per day, with a total of 400-500 eggs in their 50-day life time. Larvae feed on mealybugs for 12-17 days, and then pupate. A single larva can consume 250 small mealybugs. Immature "Crypt" beetle larvae look like large mealybugs with a similar white, waxy coating – keep an eye for them as this is a good sign of a growing predator population. Adults emerge in 7-10 days, mate and females begin laying eggs in 5 days. Adults and young larvae prefer to eat mealybug eggs, but older larvae feed on all stages of mealybugs.

#### COMPENDIUM OF COTTON MEALYBUGS

## 24. PREDATORY POTENTIAL OF SPALGIS EPIUS

Lepidopteran predator, *Spalgis epius* (Lycaenidae: Lepidoptera) is a well known butterfly feeding on various species of pseudococcidae. *S. epius*, being the dominant predator, feeds efficiently on the ovisacs, nymphs and adult of *P. marginatus*. Newly hatched larvae of *S. epius* are pale pink in colour and remain inside the mealybug ovisac devouring the eggs of the mealybug. Natural occurrence *S. epius* was recorded on *P. marginatus* on cotton. Second, third and fourth instar larvae of *S. epius* were tested for the predatory potential against *P. marginatus* in the lab. Among the 3 stages of the predator larvae, 3<sup>rd</sup> instar larvae of the predator, 3<sup>rd</sup> instar predated significantly maximum number of egg masses of 9.6 / day as compared to 2<sup>nd</sup> instar (6.3).

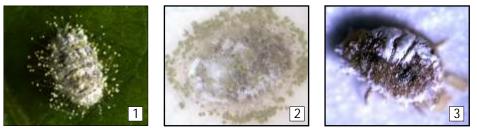


1. Caterpillar feeding on crawlers, 2. Mealybug colonies after predation, 3. Pupa, 4. Adult The total life cycle lasts for  $14.83 \pm 0.44$  days with the larval life span of  $9.83\pm2.39$  (I instar:  $2.5\pm0.5$  days; II instar :  $1.75\pm0.25$  days; III instar :  $1.41\pm0.38$  days; IV instar :  $2\pm0.5$  days; V instar :  $2.1\pm0.76$  days). The larvae pupate on the under surface of the leaves to form the characteristic rhesus monkey face chrysalis. The pupal period is about  $5.45\pm0.50$  days. Fifth instar larvae consumed as much as 18 to 26 ( $22.33\pm3.21$ ) ovisacs and 112 to 132 ( $121.66\pm8.86$ ) nymphs and adults of the mealybugs. During the whole larval period the predatory larvae devoured about 42 to 53 ( $48.15\pm4.08$ ) ovisacs and 196 to 222 ( $210.99\pm10.77$ ) nymphs and adults of *P. marginatus* (Thangamalar *et al.*, 2010).

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### 25. ENTOMOPATHOGENIC FUNGI ASSOCIATED WITH MEALYBUGS

Entmopathogenic fungi in the genus Fusarium, Entomopthora, Paecelomyces, Verticillium lecanii, Metarrhizium anisopliae, Beauveria bassiana are prevalent in the humid months, these fungi plays role in the control of mealybug in the cotton ecosystem. Commercial formulations of some of the fungi like Verticillium lecanii, Metarrhizium anisopliae, Beauveria bassiana are already available in the market.



Infected P. solenopesis by 1. Fusarium sp., 2. Metarrhizium anisopliae 3. Beauveria bassiana

Pathogenicity of some Entomopathogenic fungus

 $LD_{50}$  and  $LT_{50}$  were calculated for fungal pathogens viz., *Metarrhizium anisopliae*, *Verticillium lecanii* and *Beauveria bassiana* against first, second and adult of cotton mealybugs viz., *P. marginatus* and *P. solenopsis*.  $LD_{50}$  and  $LT_{50}$  values of fungal pathogens are given in Table 4.

Table 4.  $LD_{50}$  and  $LT_{50}$  of fungal pathogens for mealybugs

Fungal pathogens	P. solenopsis			P. marginatus		
	1 <sup>st</sup> instar	2 <sup>nd</sup> instar	Adult	1 <sup>st</sup> instar	2 <sup>nd</sup> instar	Adult
Metarrhizium anisopliae						
LD <sub>50</sub> (Spores/ml)	8.7x 10⁵	1.3x 10 <sup>6</sup>	5.4x10 <sup>6</sup>	5.0x 10⁵	9.8x10⁵	1.3x 10 <sup>6</sup>
LT₅₀ at 10 <sup>5</sup> (days)	4.50	5.97	6.27	4.20	5.03	6.00
LT <sub>50</sub> at 10 <sup>6</sup> (days)	4.47	5.67	6.24	3.99	4.89	5.77
LT <sub>50</sub> at 10 <sup>7</sup> (days)	3.94	5.45	6.22	3.56	4.87	5.66
Beauveria bassiana						
LD <sub>50</sub> (Spores/ml)	9x10⁵	3.9x10 <sup>6</sup>	5.3x10 <sup>7</sup>	8.2x10⁵	2.5x10 <sup>6</sup>	1.4x10 <sup>7</sup>
LT <sub>50</sub> at 10 <sup>5</sup> (days)	4.95	6.10	7.17	4.68	6.00	7.02
LT <sub>50</sub> at 10 <sup>6</sup> (days)	4.60	5.89	6.83	4.71	5.37	6.80
LT <sub>50</sub> at 10 <sup>7</sup> (days)	4.09	5.60	6.71	3.88	5.19	6.52
Verticillium lecanii						
LD <sub>50</sub> (Spores/ml)	1.5x10⁵	3.2x10 <sup>6</sup>	1.3x10 <sup>7</sup>	5.9x10⁵	1.7x10 <sup>6</sup>	1.2x10 <sup>7</sup>
LT <sub>50</sub> at 10 <sup>5</sup> (days)	5.72	6.47	7.22	5.46	6.21	6.93
LT <sub>50</sub> at 10 <sup>6</sup> (days)	5.35	5.99	7.07	4.82	5.57	6.98
$LT_{50}$ at 10 <sup>7</sup> (days)	4.79	5.65	7.07	4.55	5.22	6.96

#### COMPENDIUM OF COTTON MEALYBUGS

## 26. MEALYBUG HOST PLANT MANAGEMENT AS A CULTURAL COMPONENT

Discouraging weeds viz., Acmella uliginosa (SW.) Cass., Helianthus sp., Parthenium hysterophorus Linn., Pentanema indicum (L.) Y. Ling, Taraxacum officinale Linn., Xanthium strumarium Linn. (Asteraceae); Abelmoschus ficulneus (Linn.), Abutilon indicum Sweet, Malvastrum coramandelinum G., Sida cordifolia Linn., Boerhavia diffusa Chois. (Malvaceae); Euphorbia heterophylla Linn, Euphorbia hirta Linn., Phyllanthus amarus Linn. (Euphorbiaceae); Corchorus trilocularis Linn., Triumfetta rhomboidea Linn. (Tiliaceae), Trianthema portulacastrum Linn. (Aizoaceae), Asteracantha longifolia Nees (Acantheceae), Digera muricata (Linn.) (Amaranthaceae), Clitoria ternatea Linn. (Fabaceae), Boerhavia diffusa Chois. (Nyctaginaceae) and Portulaca oleracea Linn. (Portulacaceae) during cotton season as well as off-season as a part of cultural component of Integrated Pest Management holds the key in the mealybug suppression besides avoiding cultivation of other malvaceous and solanaceous crops in the vicinity of previously mealybug affected fields. Timely suppression of preferred weed hosts will not only reduce the dependency on the chemical pesticides but also has wider appeal and bears cascading effect on the ecosystem that harbours beneficial parasitoid like A. bambawalei which has very good potential besides other naturally occurring bio agents.

Pigeon pea [*Cajanus cajan* (Linn) Mill.] which falls into grade-1 (least preferred) host plant category in cotton cropping system has been suggested by Nagrare *et al.* (2009) to be cultivated at border and intercrop, holds good in restricting movement of *P. solenopsis* especially in central zone beside other advantages like pigeon pea act as a refugee to American bollworm *Helicoverpa armigera* for delaying resistance development in vast cultivated Bt-cotton (>95%), compatible in cotton cropping system owing to its identical production practices and also nutritional security of the countrymen. Thus promoting cultivation of pigeon pea at border 2 rows all around the field and 2 rows intercropped after 6-8 rows of cotton wherever feasible would immensely help in preventing spread of *P. solenopsis* population.



1. Intercropped pigeon pea in early phase of growth, 2. Pigeon pea at reproductive stage

## 27. EFFICACY OF BIO FORMULATIONS AND **INSECTICIDES AGAINST P. SOLENOPSIS**

Mortality of *P. solenopsis* against Bio-formulations and Insecticides under Lab conditions.

SI No	Insecticide	Trade name	Dose	4 DAT 8 DAT	
	Biorationals				
1.	Verticillium lecanii	Vercitile	10 gm/l	55.09 (47.92)	61.20 (51.47)
2.	Beavaria bassiana	Myco-Jaal 10% SC	2 ml/l	12.12 (20.37)	55.02 (47.88)
3.	Neem oil 0.03 % EC	Neemgold	50 ml /l	75.17 (60.11)	77.13 (61.43)
4.	Herbal product	Cal MB	1 ml/l	53.04 (46.74)	72.00 (58.05)
5.	Bacteria <i>Photorhabdus</i> <i>luminescens</i> sub spp. Akhurstii (Strain K-1) Nitrosoguanidine	Bioprahar	5 %	1.52 (7.09)	19.29 (26.05)
6.	Imidacloprid 17.80 % SL	Confidor®	20 g a. i./ ha	56.25 (48.59)	74.00 (59.34)
7.	*Thiomethoxam 25 % WG	Battalion	100 g a. i./ ha	61.20 (51.47)	78.21 (62.17)
8.	*Acetamiprid 20 % SP	Starattack	20 g a. i./ ha	79.01 (62.73)	86.06 (68.08)
9.	Thiodicarb 75 % WP	Larvin	1500 g a. i./ ha	76.48 (60.99)	95.05 (77.14)
	Cyclodiene				
10.	Endosulfan 35 % EC	Endoin®	525 g a. i./ ha	50.99 (45.57)	56.01 (48.45)
	Insect Growth Regulators				
11.	*Novaluron 10 % EC	Rimon®	50 g a. i./ ha	16.99 (24.34)	31.98 (34.44)
12.	Buprofezin 25 % SC (U)	Applaud®	100 g a. i./ ha	16.14 (23.69)	64.32 (53.32)
13.	*Flubenoxuron 10 % DC	Cascade 10 DC	100 g a. i./ ha	20.99 (27.27)	37.95 (38.03)
	Oxadiazine				
14.	*Indoxacarb 14.5 % SC	Avaunt <sup>™</sup>	75 g a. i./ ha	36.97 (37.45)	46.95 (43.25)
	Spinosyns				
15.	Spinosad 45% SP (U)	Spintor	75 g a. i./ ha	50.00 (45.00)	76.01 (60.67)
	Organophosphate				
16.	Chlorpyriphos 20 % EC	Starban	250 g a. i./ ha	90.01 (71.57)	100.00 (90.00)
17.	Quinalphos 25% EC	Starlux EC25	500 g a. i./ ha	86.26 (68.24)	96.26 (78.85)
18.	Monocrotophos 36% SL	Monohit <sup>™</sup>	2 ml/l	71.13 (57.50)	90.90 (72.44)
19.	Profenophos 50% EC	Prabal	1000 g a. i./ ha	75.17 (60.11)	100.00 (90.00)
20.	Acephate 75 % SP	Asafat	292 g a. i./ ha	91.03 (72.57)	96.26 (78.85)
21.	Oxydematon methyl 25% EC	Metasystox	2 ml /l	11.30 (19.64)	53.00 (46.72)
22.	Triazophos 40 % EC	Hostathion	500 g a. i./ ha	88.08 (69.80)	98.99 (84.23)
23.	Methyl parathion 50 % EC	Dhanumar 50	1 ml/l	93.04 (74.70)	97.09 (80.17)
24.	Dimethoate 30 % EC	Rogor	200 g a. i./ ha	76.01 (60.67)	97.43 (80.78)
25.	Dichlorovos 76 % EC	Nuvon®	500 g a. i./ ha	56.99 (49.02)	79.32 (62.95)
	Synthetic Pyrethroids				
26.	Cypermethrin 10 % EC	Bilcyp®	60 g a. i./ ha	21.97 (27.95)	60.00 (50.77)
27.	Fenvelerate 20 % EC	Fenvel®	100 g a. i./ ha	4.72 (12.55)	35.00 (36.27)
28.	Deltamethrin 2.8% EC	Desis	12.5 g a. i./ ha	5.28 (13.28)	29.82 (33.10)

\*Not classified, U-Unlikely to present acute hazard in normal use

[The WHO Classification of Pesticides by Hazard WHO: Class Ia-Extremely hazardous, Class Ib- Highly hazardous, Class II- Moderately hazardous, Class III- Slightly hazardous Green]

## **Bio-formulation Developed By CICR**

Bio-formulations 'Mealy-Quit' and 'Mealy Kill' have been developed. 'Mealy-Quit' have been evaluated at different locations of TMC MM I during 2008-09. 'Mealy-Quit' and 'Mealy Kill' have been tested under AICCIP during 2009-10, both were found effective against mealybug *P. solenopsis*.

Efficacy of Bio formulations against P. solenopsis under filed conditions

Population reduction of Mealybugs by different insecticides and bio formulations under field conditions at Lam Guntur is given in Table 5.



S. No.	Particulars	Mealy bug incidence (Grade)		
		Before spray	After spray	
1	Acephate @ 1.5 g / I	2.83 (1.95)	0.16 (1.07)	
2	Chlorpyriphos @ 3 ml / I	3.16 (2.03)	0.33 (1.15)	
3	NSKE @ 50 ml / I	2.66 (1.90)	3.00 (2.00)	
4	Neem oil @ 5 ml / I + Nirma Powder @ 1g / I	2.5 (1.86)	2.5 (1.86)	
5	Nirma powder @ 1g / I	2.5 (1.86)	3.33 (2.07)	
6	Verticillium lecanii @ 5 ml / l	2.6 (1.90)	3.66 (2.15)	
7	Metarhizium anisopliae @ 5 ml / l	2.16 (1.77)	4.00 (2.23)	
8	Baeuveria bassiana @ 5 ml / l	2.33 (1.82)	4.00 (2.23)	
9	Fish oil rosin soap @ 25ml / I	2.83 (1.95)	3.33 (2.08)	
10	Mealy quit @10ml / I	2.5 (1.86)	0.5 (1.21)	
11	Bugnil @ 2.5 ml / I	2.6 (1.91)	0.5 (1.21)	
12	Control (Water spray)	2.16 (1.77)	3.66 (2.15)	
	T-test	NS	Sig	
	SEd	0.13	0.12	
	CD (P=0.05)	NS	0.26	
	CV%	8.96	9.0	

#### Table 5 : Efficacy of bioformulation against *P. solenopsis*

## 28. PACKAGE OF PRACTICES FOR MANAGING MEALYBUGS ON COTTON

#### Tips for management

It is important to remember that mealybug crawlers spread through human interventions such as spraying, irrigations, frequent movement through the infected area etc. Therefore avoid disturbing mealybug affected plants. It is important to remember that young cotton plants can overcome mealybugs and it is better not to resort to chemical sprays on young plants that have slight infestation of the mealybugs in early vegetative stages of the crop. It has been observed that the mealybugs were unable to establish colonies on the cotton crop during early vegetative and peak vegetative stages. It is only in rare cases, which is generally possible on a few susceptible genotypes, that mealybugs colonize plants during vegetative stage.

All over the country, several parasitoids (predominantly *Aenasius bambawalei*.) and coccinellid beetle predators are now found to keep mealybug populations under control, thereby preventing spread and damage. Insecticides such as profenophos, chlorpyriphos, monocrotophos etc. which are being commonly used for mealybug control, destroy the parasitoids and predators and can result in mealybug outbreaks. Therefore, insecticide applications should be avoided until peak boll formation stage, so as to allow further establishment of the parasitoid and predator complex in the ecosystem. Eco-friendly insecticides such as neem oil based botanicals and buprofezin can be used if necessary in the initial stages so as to keep mealybugs under check while causing minimum disturbance to the ecosystem.

However during peak boll formation stage, mealybugs can establish colonies but are initially restricted to a few plants along the border rows, adjacent to the source of infestation and thus can be effectively managed through early detection and initiation of interventions to control early stages of infestation. If timely scouting and appropriate control measures are not initiated cotton crop is likely to be severely damaged with mealybugs.

Insecticides should not be applied all over the field to manage mealybugs. Such a practice disrupts the ecosystem and does not allow naturally occurring parasitoids and predators to establish natural control. Therefore, the following practices are advised:

- Locate infested plants with more than one twig infested completely with mealybug colonies.
- Do not allow physical contact with the infested plant. Do not disturb the plant vigorously. If possible, the affected twig can be gently detached from the plant, collected in plastic bags and take far away from fields and destroy by burning.
- If at least 10% infested plants exceed grade-2 (at least one stem completely colonized with mealybugs) in more than 40 plants randomly sampled plants per acre, chemical control measures may be initiated.
- Insecticide application should start first on the neighboring plants and then as spot application near the root zone, base of the plant and other infested parts.

	Why not to do		Ratoon crop offer food and shelter for mealybugs and provides inoculum for next season.	Mealybug populations survive on stalks and pass on to the next season.	Mealybug spread through water. If weeds are destroyed mealybugs move to the adjacent crop.	Fuzzy seeds may
n cotton	What not to do		Ratoon cropping or allowing the cotton crop to continue to stand in the field after the final harvest.	Stacking of cotton stalks in or nearby areas of the fields	Do not allowo infested plants in to irrigation canal. Do not spray only weedicides.	Using fuzzy seeds
ing mealybug o	How to do		Removal of cotton crop from the fields immediately after the last picking and maintenance of host free period.	The dry cotton stalks should be pulled out of the fields or shredded and burnt off in situ before ploughing the field.	Biological- inoculative release of Zygogramma bicolorata @ 500-1000 beetles /ha on parthenium grass. Mechanical- Hand weeding and destructions Spray weedicide + insecticides on bunds.	Delinting should be
Do's and Don'ts for managing mealybug on cotton	Why to do		To prevent continuous food supply and shelter for multiplication and carryover of mealybugs.	Destruction of cotton stalks following harvest reduces the shelter and food supply to mealybug and carryover to next season.	Weeds especially congress grass and Xanthium are the most suitable hosts for mealybugs and assist them to survive and spread on the adjacent crop.	Delinted seeds
DO S and DO	When to do		Immediate after last picking and between two cropping seasons	After final picking is over.	During the crop season and off-season.	A the time of sowing/
	What to do	Cultural practices	Early crop termination	Destruction of cotton stalks.	Clean cultivation: Destroy alternate weed host growing on field bunds, water channels and wastelands in the area.	Use acid delinted

harbor infective stages

for sowing.

acid washed with water

infective stages of

the mealybugs.

do not carry any

planting

seeds for sowing

done with sulphuric

neutralized with lime

and dried under shade

of mealybug, especially crawlers.

Nondescript cultivars

Use of nondescript or

F2 Bt hybrids.

CICR while making a

Consult the SAUs/

Approved varieties/

hybrids are tested oefore release in

Before planting and procurement of seed material.

Select varieties/ hybrids approved by GEAC or SAUs of that zone.

choice of genotype.

oarticular zone for their tolerance to pest and other abiotic factors.

may be susceptible to mealybugs.

Do's and Don'ts for managing mealybug on cotton

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Why not to do	Malvaceous and solanaceous crops are good hosts for mealybugs. They serve as shelter and spread mealybug infestation	Mealybugs spread through water, air, human, animal, farm implements etc.		Use of insecticides disrupts native predators and parasitoids.	
What not to do	Avoid growing malvaceous and solanaceous crops near the cotton fields.	Do not allow free movement of labour/ animals in infested fields		Do not use chemical insecticides at early stage of crop.	
How to do	Growing two rows of densely planted pigeonpea or maize or bajra around the cotton fields and also if possible as intercrop of 1- 2 rows after 5-6 rows of cotton.	By weekly observations, the pest can be effectively managed through early detection and initiation of interventions to manage early stages of infestation.		Spray on the crop adjacent to the infested plants and at the base of the infested plants without disturbing the mealybug colonies.	Release the adult beetle during morning or evening hours to avoid direct exposure to hot sunlight.
Why to do	These crops offer least support for the growth and multiplication of mealybugs. Border rows act as barrier crop that prevent mealybug infestation from border weeds.	The pest is initially restricted to a few plants along the border rows, adjacent to the source of infestation.		Spot application restricts the spread of mealybugs. These formulations are less harmful to natural enemies and thus help in conserving ecosystem.	Cryptolaemus montrouzieri, is a naturalized predator of mealybugs & feeds voraciously on P. solenopsis.
When to do	At the time of planting.	After the sowing of cotton crop	jical control	Initial stage of infestation. When 1-2 infested plants observed of grade -2 (at least one stem completely colonized with mealybugs) in more than 40 plants per acre	Inoculative releases of the ladybug beetle, prior to the cotton season, on weeds le. and perennial trees harbor mealybug colonies, and also on infested cotton plants.
What to do Cultural machices	Grow pigeonpea, bajra or maize as border crop wherever possible.	Regular monitoring of the pest.	Botanical and Biologic	Neem Seed Kernel Extract (NSKE 5%) 50ml/I + Neem oil 5ml/I + detergent powder 1gm/I can be sprayed as spot application on infested stalks. Fish oil rosin liquid 10ml/I or Karanj oil 10ml/I or Karanj oil	Use of Cryptolaemus montrouzieri adults /grub@ 10 per infested plants wherever available

Why not to do		Fungal spores germinate and cause disease in the insect when optimum relative humidity (>60%) conditions prevail.		Insecticides with high ecotoxicity should be avoided since they are not only ecologically hazardous, but also detrimental to predators & parasitoids wasps that control mealybugs and other insect pests. FAO specifically recommend class WHOTa and WHOTb insecticides for not using in developing countries.				
What not to do		Do not use pathogens formulations Verticillium lecanii and Beauveria bassiana during other months when humidity is low.		Avoid use of insecticides with high eco-toxicity such as methyl parathion, (classified by the World Health Organization (WHO) as WHO 1a: extremely hazardous), dichlorvos, methomyl, triazophos and metasystox and monocrotophos, (WHO 1b: highly hazardous).				
How to do		Spray of biopesticides formulations during morning / evening hours on infested crop area.		Spray the chemicals first on plants around infested plants and then as spot application at the infested plants.				
Why to do		The formulations disrupt growth and multiplication of mealybugs by causing disease without harming to the other natural enemies and the environment.		WHO class III (Slightly hazardous) Acephate, Malathion and WHO Class IV (Unlikely hazardous) Buprofezin cause less harm to the environment.	WHO class II (Moderately hazardous) Quinolphos, Chlorpyriphos, Profenophos Thiodicarb cause comparatively less harm to the environment.			
When to do	ogi zal control	Initial infestation during August-October i.e high humid months coinciding with vegetative growth phase of crop.		When 10% infested plants observed of grade-2 (at least one stem completely colonized with mealybugs) in more than 40 plants per acre	When > 10% infested plants observed to exceed grade 2 infestation in more than 40 plants per acre.			
What to do	Botanical and Biologi	Spray biopesticides viz., Verticillium lecanii (Potency 2 X 10°C.F.U /gm) 10gm/l and Beauveria bassiana (Potency 10° spores/m) 10ml/l.	Chemical control	Spray less hazardous insecticides, such as Acephate, 75 SP 1gm/l, Malathion 50 EC 2m/l, Buprofezin 25 SC 1m/l	As the last option, spray moderately hazardous insecticides:Ouinolphos 25 EC 5.0 ml/l Chlorpyriphos 20EC 3.0ml/l Profenophos 50EC 5.0 ml Thiodicarb 75WP 5.0gm/l			

(Available at: www.cicr.org.in)

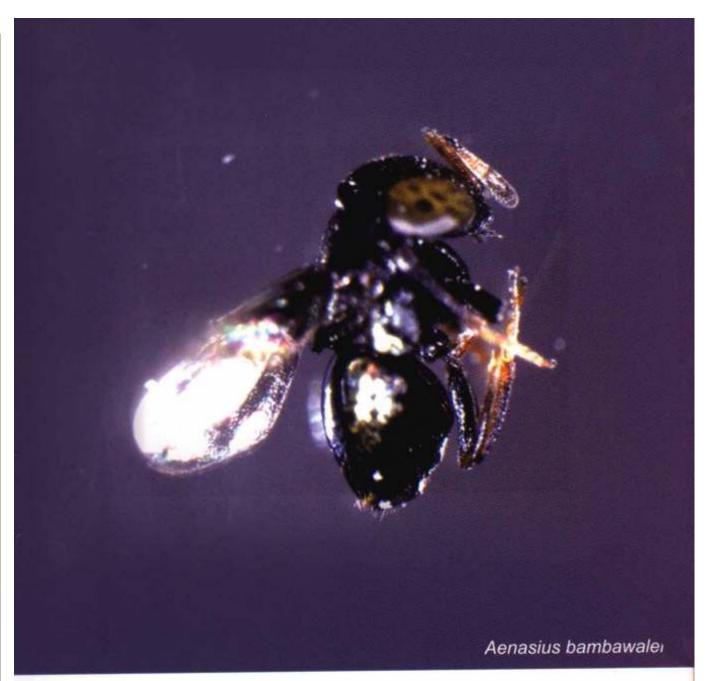
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