Journal of Phytology 2010, 2(7): 28-36 **ISSN: 2075-6240**
An Open Access Journal Available Online: www.journal-phytology.com

REGULAR ARTICLE

AGONISTIC ASSOCIATION OF LEPIDOPTERA AND FUNGUS IN THE DEVELOPMENT OF LEAF-SPOT DISEASE IN HIGH ALTITUDE MANGO AND ITS CONTROL

Arup Kumar Mitra*, Pip Banerjee, Gaurav Saha, Priyanka Lahiri, Ishita Mukherjee, Ayantika Bhattacharya

Department of Microbiology, St. Xavier's College, 30, Park Street, Kolkata, West Bengal 700016, India

SUMMARY

Plants are common prey for pests, though plants at high altitudes are less prone to diseases. However, our sample proved to be an exception, as disease in plants have become a major problem in North India, especially in old, crowded orchards where there is excessive shade .Mango, our test plant, is well adapted to tropical and subtropical climate. Here we considered the mechanism of disease initiation in the mango leaves by the entry of a fungal pathogen- *Cercospora mangiferae*, and its possible agonistic association with an insect of the Lepidoptera group, *Procontarinia* sp . Our aim is to suggest a pesticide to avert the entry and reduce the occurrence of the disease. The specimen, collected from a place called Jorolle (NH 88) near Sundernagar, is 10 kms away from the Beas-Sutlej confluence in the state of Himachal Pradesh, during the months of January-February, the temperature recorded was between 7-14°C. The environment in the vicinity of the mango orchard was dry, windy, and grimy and plagued by vehicular emissions. There were predominantly 2 kinds of leaf spots-a white and a brown spot. The spread of the disease started from the lower mature leaves to the upper younger leaves. Enormity of the infection was much greater in leaves having galls along their margins. The gall formation results due to the mechanical damage caused by the infection due to a midge fly (*Procontarinia* sp). The average diameter of galls ranged between 3-4mm. As affirmed earlier, the leaves with large number of galls are the primary home for the fungus- *Cercospora mangiferae* where they reside in larger numbers. Although the mechanism of an agonistic association is obscure but the possibility of such an association cannot be ruled out completely; where the primary infection caused by the midge insect paves the way for secondary infection by the fungus. Our sole intention was to prevent occurrence of such an association, by inhibiting both the infections from occurring individually. Our test pesticide belonged to the Malathione group. Its main component is monocrotophos which interferes not only with the nerve impulse transmission of the insect but also damages the cell wall of the fungal pathogen thereby attending both the problems. The experiment was performed with different concentrations of pesticide and it was observed that at 43.5%w/w it was effective enough to prevent 100% germination. Our studies provide a conclusive result which suggests that if the pesticide, at the effective concentration is sprayed till run-off, the young tender leaves of *Mangifera indica* will be protected from both the midge insect as well as the fungal pathogen.

Key words: Chausa, Langra, Dashehari, Leaf spot, *Cercospora mangiferae*, White spot, Brown Spot, Gall, *Procontarinia* sp., pesticide, Malathione, Hilcron, Monocrotophos

Arup Kumar Mitra et al. Agonistic Association of Lepidoptera and Fungus in the Development of Leaf-spot Disease in High Altitude Mango and its Control. J Phytol 2/7 (2010) 28-36.

*Corresponding Author, Email: drakmitra01@hotmail.com

1. Introduction

Mango or *Mangifera indica* which belongs to the family Anacardiaceae, grows well in tropical regions with a distinct dry and wet season. In India where 40 per cent of total

fruits grown is mango, it is an important fruit crop similarly in other tropical and subtropical countries of the world. Although India is the largest producer of mango but in terms of productivity, it ranks sixth. The low productivity is mainly due to the associated disease problem. Mango is affected by a number of diseases at all stages of its development. These diseases manifest themselves as several kinds of rot, die back, mildew, necrosis, scab, blotch, stem bleeding, wilt, spots, canker, sooty mould and malformation [Prakash Om, 1991]. Some of the fungal diseases are:Anthracnose, caused by *Colletotrichum gloeosporioides*, poses serious problems for the export quality of the fruit as it is the most serious and most widespread disease of mango. Symptoms include brown or black necrotic lesions on the leaves, shoots and flowers, and sunken brownish-black spots on the fruit; Powdery mildew, caused by *Oidium mangiferae*, occurs specifically in regions where flowering takes place during the hot dry season with a rather high relative humidity .The fungus attacks the floral buds, young fruit and young leaves; it causes necrosis of infected tissues. On the leaves, Cercospora (*Cercospora mangiferae*) causes round, reddish-brown spots surrounded by a yellowish-green halo . On the fruit, the disease begins with tiny sooty spots which gradually enlarge. Treatments against anthracnose are also effective against Cercospora spot.[Popenoe W,1974]

Leaf spot diseases cause great loss and hamper the efforts made to increase the yield of mango tree. They impoverish the leaves, diminish the photo-synthetic efficiency and upset normal physiological activity of the host. Some of these diseases take heavy toll of trees, and have become limiting factor in mango cultivation in some regions. Bloom blight or Blossom blight in some years causes a complete failure of the crop.[Prakash Om,1991].Recent studies like [Harris K.M. and Schreiner I.H.,1991] and [Sankaran T, Mjeni A.M. 2009] suggest wide spread diseases caused due to fungal pathogens and role of insects of the Lepidoptera group in such infections of mango plants. According to Harris et al(1991) a new species of gall midge, *Procontarinia schreineri* attacks mango foliage in Guam. According to this report eggs are laid on young mango leaves and larvae, which develop rapidly over about 5 days, induce blister galls before leaving to pupate. Secondary damage to infested foliage is caused by the fungus *Colletotrichum gloeosporioides*, which invades damaged leaf tissue and causes mango anthracnose disease .Another interesting observation of this report was that the main factors affecting populations were rainfall and location, this was noted by studying *P. schreineri* population fluctuations on mangoes over three years by counting the numbers of galls. More galls were present during rainy periods, possibly because high humidity improves larval and pupal survival. Gall populations were generally low and unlikely to have direct effects on fruit yields but the introduction of new improved varieties of mango might increase susceptibility to damage. In addition, old galls on damaged leaves may provide reservoirs of anthracnose inoculum. Further according to Sankaran et al (2009) investigations in India, where the midge(*Procontarinia matteiana)* is one of several native pests of mango, have led to the discovery of a complex of hymenopterous parasites of *P. matteiana* and allied species. A number of insects belonging to the Procontanaria group affecting mango plants are *Procontarinia allahabadensis ,P. amraeomyia,P. biharana, P. brunneigallicola, P. echinogalliperda,P. keshopurensis,P. mangiferae, P. mangifoliae ,P. matteina ,P. tenuispatha ,P. viridigallicola* [Sharma R.M ,2009]

The chemical based strategies have been so far dominating for management of mango diseases but it has caused serious imbalance in the agro-ecosystem. To control diseases in mango plants mostly chemicals are used. Strangely, about 70 percent of the amount of sprayed chemicals does not stick to the plants. Enormous quantities of chemicals that fall on to earth get mixed up with soil adversely affect microbial life. Some problems like non-target effects of chemicals as well as chemical induced diseases are being experienced. A shift towards non-chemical strategies like IDM is likely to correct the imbalance in our

approach.[Prakash Om ,1991] A group of chemicals that is used for this purpose is malathione.

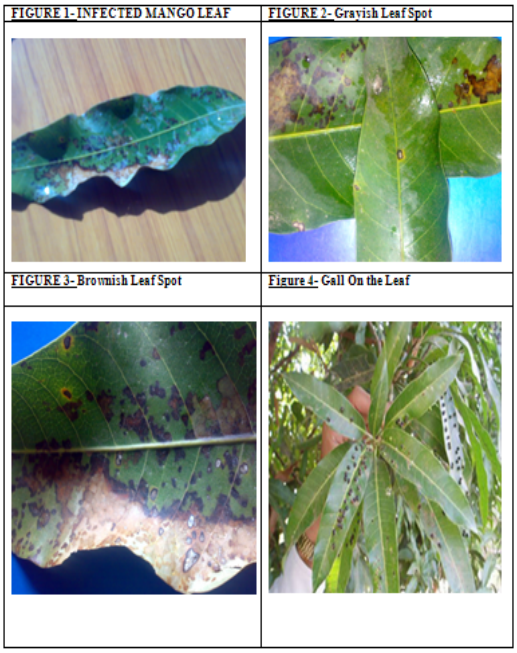
Malathione is the most commonly used insecticide in the U.S.; the U.S.Environmental Protection Agency (EPA) estimates that annual use of malathione is over 30 million pounds. About 60 percent of this is used in insect eradication programs (for boll weevils, grasshoppers , and fruit flies). It is also used on a variety of food crops, for mosquito control, in home yards and gardens, and to kill head lice. Malathione kills insects because it is converted inside animals into malaoxon a chemical relative that inhibits an important central nervous system enzyme called acetylcholinesterase (AChE) , which is involved with the transmission of nerve impulses.[Sankaran T., Mjeni A.M. ,2009] When this enzyme is inhibited, the transmission system "jams," resulting in restlessness, hyper-excitability, convulsions, paralysis, and death. All insecticides in the organophosphate chemical family share a similar mode of action [Journal of Pesticide Reform 2003]. In mammals, malaoxon has similar effects on AchE. However, in mammals AChE is not used in the central nervous system, but rather in nerves that connect with muscles. This means that symptoms in mammals are different than those in insects [Journal of Pesticide Reform, 2003]

Thus in this study we have collected samples (mango leaves) from Jorolle (NH 88) [near a place called Sundernagar 10 kms away from the Beas-Sutlej confluence in the state of Himachal Pradesh] in the month of January-February when temperature was around 7-14°C and the place was dry, dusty and plagued by vehicular emission. Then we studied the leaf spots and galls and isolated the organism causing it whose results have been tabulated below. Further a pesticide was proposed and its effective concentration was also determined.

2. Materials and Methods

I. **Isolation Of Leaf Fragments With Leaf Spot Symptoms –** Our mango leaf sample (Refer to **Plate 1, Figure 1**) had primarily two kinds of leaf spots. -a grayish leaf spot (Refer to **Plate 1, Figure 2**) and a brownish leaf spot (Refer to **Plate 1, Figure 3).** The leaf spots were isolated by cutting leaf fragments, and placing in a sterile petri plate containing 0.1% $HgCl_2$ (mercuric chloride) for surface sterilization.

Plate 1



II. **Isolation Of The Gall- (Refer to Plate 1, Figure 4**) Mango galls were also present and were prevalent along the margins of the infected leaf.They were placed in a sterile petri-plate containing 0.1% $HgCl_2$ (mercuric chloride). The mercuric chloride served the same purpose, which is surface sterilization.

III. **Isolation Of The Fungus –** Leaf fragments containing the spots, after surface sterilization were placed in a PDA (potato dextrose agar) slant. They were then incubated in BOD (25^0C-26^0C) for five to six days.

IV. **Identification Of Organism -** The PDA slant showed fungal growth. Then staining was performed to identify the type of fungus. Since it is a fungus, we performed lactophenol cotton blue staining.

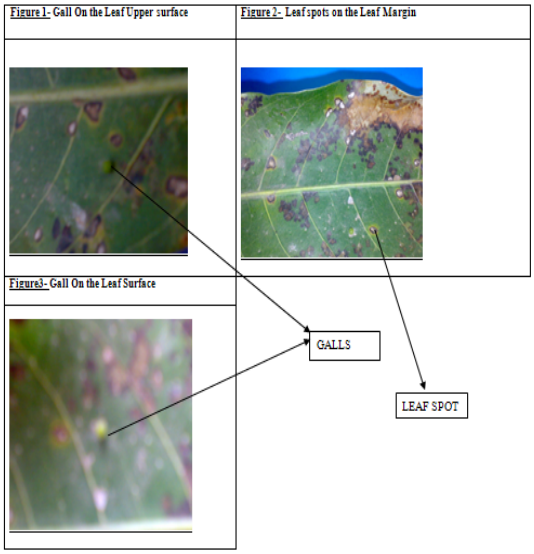
After performing the staining procedure, we identified the organism to be *Cercospora mangiferae* through its micro and macro conidial character.

V. **Creation Of A Pure Culture-** Pure culture of the fungus was done that was obtained in PDA by taking small amount of the fungal mass and plating it again in a PDA petri-plate and incubating at room temperature for five to six days under sterile conditions.

VI. **Micrometric Measurement Of Conidia –** The principle of micrometry was followed and we determined the dimensions of the micro and macro conidia of the fungal pathogen. (Refer to **Table II**)

VII. **Transverse Section Of The Gall –** Transverse section of the isolated gall was observed under the microscope to determine the identification of the midge insect. It was found that the larva was situated inside the gall. A picture was taken of the larva inside the gall. (Refer **to Plate 3 Figure 3)**

Plate 2



VIII. **Determination Of The Effective Concentration Of The Pesticide -** The common pesticide used is Monocrotophos.The marketed concentration is 36%w/w. We prepared two concentrations, one of the marketed concentration and the other was half diluted version of the marketed form, the concentration being 18%w/w. The result of percentage sporulation has been given in a table form (Refer to **Table V**). Ultimately the effective concentration of the pesticide is determined by unitary method.

3. Results

Comparative study of the leaves infected by Midge insect, *Cercospora mangiferae* or both

There were many mango plantations, out of which we chose 1 tree from 1 plantation and studied 150 matured leaves. We then determined the number of leaves that were infected and not infected. We also found how many leaves were infected by the insect only; by the fungal pathogen only and by both. The result that we obtained helped us establish the fact that an agonistic relation exists between both the insect and the fungal pathogen. A pie chart has been provided to represent the table in a statistical manner (Refer to **Plate 4**).

Plate 3

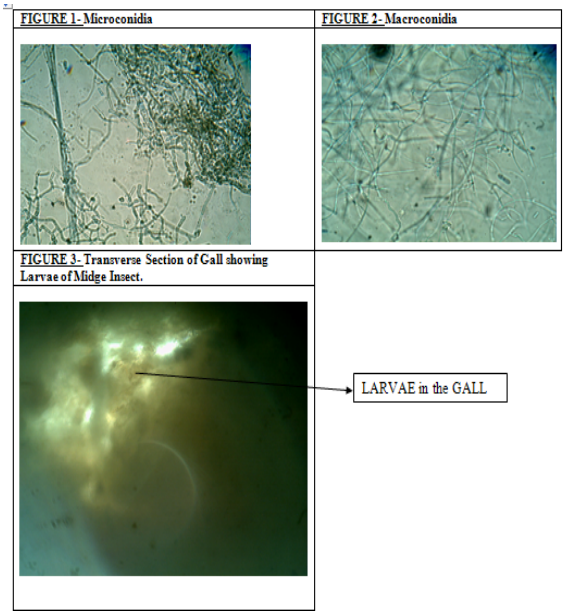


Plate 4: Pie chart

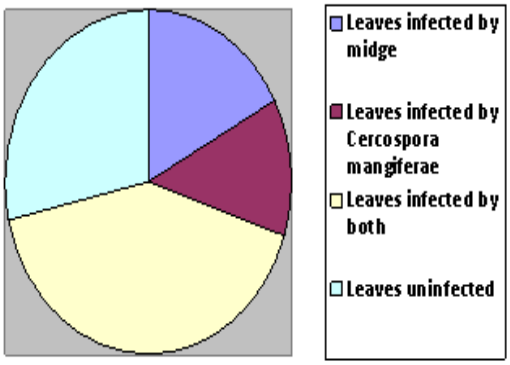


Table I- Comparison of No. of Leaves infected by Midge and *Cercospora mangiferae**

Study of Leaves	Leaves Infected by Midge insect only	Leaves Infected by Cercospora mangiferae only	Leaves Infected by both Midge and Cercospora mangiferae	Leaves intact (not infected with either of the pathogen)
NUMBER	26	19	62	43
PERCENTAGE	17.3	12.7	41.3	28.7

*Total Number Of Leaves Studied = 150

Characteristic feature of the fungal pathogen

The principle of Micrometry was followed to measure the dimensions of the micro and macro conidia. The measurement of the micro and macro conidia has been provided in Table II. The picture of the micro and macro conidia has been given in Plate 3, figure 1 and figure 2 respectively.

There were two kinds of leaf spots produced, namely a brown and a white leaf spot. The dimensions of both kinds have been determined and placed in Table III.

TABLE II- Determination of the Dimensions of Microconidia and Macroconidia of *Cercospora mangiferae*

TYPES OF CONIDIA	LENGTH (μ)*	WIDTH (μ)*
MICROCONIDIA	3.85± 0.30	3.3± 0.34
MACROCONIDIA	13.2± 0.46	4.95± 0.16

* Average of 3 observations

Table III- Description of Leaf Spots formed by *Cercospora mangiferae*

TYPE OF SPOT	SPOT COLOUR	DIAMETER OF THE SPOT (mm)	MARGIN	COALESCING ASSOCIATION WTH GALL
TypeI	BROWN	2.4 ± 0.26	DARK BROWN OR BLACK	NO
TypeII	WHITE	1.9 ± 0.20	DARK BROWN OR BLACK	NO

Table IV- Determination of the Size of the Gall produced by the Midge insect and dimension of the Larva of Midge insect

NAME OF INSECT PATHOGEN	DIAMETER OF THE GALL (mm)	SIZE OF LARVA(μ)
Procontarinia spp.	2.5 ± 1	80-100

Table V- Calculation of percentage germination of the spores of *Cercospora spp.* before and after addition of the pesticide Hilcron

	No. of Spores	No. of spores germinated	Percentage Germination
1) SPORE SUSPENSION	196	98	50%
2)SPORE SUSPENSION IN EXCESS WATER	198	85	42.92%
3) SPORE SUSPENSION IN 18% CONCENTRATED PESTICIDE	194	50	25.77%
4) SPORE SUSPENSION IN 36% CONCENTRATED PESTICIDE	203	35	17.24%

Characteristic feature of the insect pathogen

The Midge insect had produced galls on the lamina of the mango leaves and its larva was residing within the gall till it goes to the next stage (Refer to Figure 3 of Plate 3). The diameter of the gall and the dimensions of the larva were determined and have been provided in Table IV.

Control of the fungal pathogen

The fungal pathogens are generally eliminated by a special type of pesticide belonging to the class monocrotophos. The marketed name is Hilcron and the concentration of Hilcron circulated in the market is 36%. This fungistatic agent performs its function by disrupting the lipid constituent of the wall of the spore. We had one control and two tests. In one test we prepared a half dilution of the marketed variety of Hilcron i.e. the concentration was half diluted to 18% while the other test was performed with the marketed concentration (36%). Then the spore germination percentage was calculated in each of the samples. The result table (Table VI) has been provided.

The pesticide also performs its regular function by keeping away the midge flies as the main constituent (Monocrotophos) acts by interfering with the nerve impulse transmission of the insect.

4. Discussion

The region of Himachal Pradesh houses many dwarf variety mango plantations. These are generally found in the high altitudes and are not of the best quality; still they are important as they serve as an important cash crop resource. The plantation where we had gone is located in the Jorolle district of Himachal Pradesh. We found that the mango trees were highly infested with two pathogens. There is a Midge insect *Procontarinia spp*. that has produced galls on the leaf while there are two different kinds of leaf spots present on the leaf caused by the fungus *Cercospora spp*. Some leaves had only midge infection that is only galls were present, while some leaves had only *Cercospora* spp. infection. But the maximum frequency was of those leaves which had both Midge insect and *Cercospora* spp. infection. This was a peculiar observation as two pathogens were sharing the same leaf which is highly unique.

The two pathogens work together to take away the nutrient content from the plant. The activity of the PSII is reduced as the areas that are located around the fungal leaf

spots have a higher temperature. This high temperature causes the stomata to close and finally the PSII's efficiency is reduced. The photosynthetic efficiency is reduced as the nutrients taken up by the plants are transported to the aid of the Midge and *Cercospora spp.* The photosynthetic efficiency being reduced, the plant's resistance decreases and more and more infection can spread after that. A hyper sensitive reaction leads to the release of ROS (reactive oxygen species) which cause further destruction of the plant cells. More and more number of PSIIs is destroyed and the photosynthetic efficiency decreases [Mihai Aldea et al.,2006]. The water and nutrient transport of the plant is altered and the pathogens receive both.

The fungal pathogen is amphigenous and mainly occurs on the leaf upper surface. The stromata of the infection generally are small, dark brown and globular. The location of the conidiophores is in the fascicles. The spore dispersal occurs by wind, rain and even by human contact. The infection basically occurs at the effective temperature of around 20°C. The mango leaf showed symptoms of circular leaf spots that had tan or gray, or even white centers. Lesions were found to be small and chlorotic. Some spots had deep brown colouration on the upper surface of the leaf. The central region of the leaf spots was observed to be grayish-white and was found to be encircled with a particularly distinct brown tissue ring. The margins of the lesions are dark brown to reddish brown [Nelson Scot, 2008]. At the initial stage, the spots appear as white but gradually turn red due to polyphenol reaction.

The other pathogen that has infected the plant leaf is *Procontarinia spp*. which is the Midge insect. They cause a kind of primary infection by performing mechanical damage to the leaf upper surface. The gall is produced by the Midge mainly in the winter season while *Cercospora* spp. is a fungus of all seasons. The insters slowly and steadily get formed along with the occurrence of voltinism. The larvae contained in the gall are very dangerous, as it parasitizes on the plant and takes away a subsequential amount of nutrients and water. So in winter the rate infection is much higher as compared to the growth rate in other seasons [Cincotta et al., 2008]

The fungal pathogen had caused a more severe infection in the leaves infested with gall .This was a peculiar thing as it was found that the primary infection caused by the midge insect helped the *Cercospora spp*. to cause secondary infection on the leaf, hence, leaf spots are produced. The midge feeds on the epidermal cells as well as causes the cortical cell degradation by penetrating into the pith region. This causes a mechanical injury to the plant and invokes the Systemic Acquired Resistance by the plant. But the plant's defence systems are rendered inactive and then further infection becomes possible. The fungus now gains a way to enter the leaf. The spores enter through a variety of dissemination processes. Then the mycelia start growing and these growing mycelia carries the larvae of the midge insect further into the plant tissues. The growing fungus releases its toxin, Cercosporin. This causes further damage to the vascular tissues by releasing Reactive Oxygen Species (ROS). The ROS causes degradation of the cell proteins and lipids leading to the vascular cell death, also the death of the vascular tissues means that the water and nutrient transport gets altered and almost all the nutrient and water gets channelized to the fungal and insect larvae pathogen. The fungal pathogen thereby provides essential nutrients and water for the larvae to consume and prosper. The fungus also helps the insect larvae to enter the vascular cells and cause the cell lysis. This is a mutualism that co-exists between the two pathogens and though we find them feeding on the same plant leaf, they share different niche. [Rohfritsch Odette,]

The toxin called Cercosporin is highly toxic and also non host specific. Hence, after its activation by sunlight, it causes the release of certain amount of ROS which changes cell polarity and this results in cell destruction. The fungal cell remains protected by the production of Vitamin B_6 which neutralizes the reactive oxygen species. Thus the fungus is able to produce leaf spots and its pathogenicity increases with the course of

time [Agrios]. This may happen in case of the Midge insect, whose cells' lipid and protein contents may be degraded by the ROS. Hence, the Midge insects have developed a new system to survive in the presence of the non host specific toxin. Midge insects release certain terepenoidal compounds which are highly aromatic and can neutralize extra charge. They react with the ROS and reduces them to their original states (singlet oxygen gets converted to molecular oxygen), thus protecting itself. But it acts only on those ROSs that have come to threaten it. The concentration of ROS when crosses a threshold level, gets neutralized by the terepenoidal compounds.

The control has become an essential thing for the mango plants to survive. They are the important cash crops of India. There are many fungicides that are useful for the control and they are Flint (strobulorin from Novartis SA (Pty) Ltd), Ortiva (strobulorin from Zeneca Agrochemicals (Pty) Ltd), Bion (salicylic acid compound from Novartis SA (Pty) Ltd),, Avogreen (Bacillus subtilis from University of Pretoria)[Duvenhage J.A.,2002], etc. but West Bengal uses the pesticide which is a Monocrotophos. The pesticide acts by inhibiting the acetylcholine transfer through the synaptic cleft. It basically destroys the enzyme choline esterase. It causes the neurotransmitter to accumulate on the synaptic junction. This results in loss of coordination in between the neurons of the nervous system and the muscles, ultimately causing paralysis of the insect [Brown Amy E.] [McCord Junior Elzie, Price James F. and Nagle Curtis A. ,2002]. It can prevent any insect belonging to the Lepidoptera class from attacking and destroying the plant. It has the ability of preventing spore germination as it has been proved by our experiment where we have calculated the sporulation percentage of the various concentrations of the pesticide [Karima, Haggag H.E. and Sayeda, Farghaly F,2007]. Spore germination can be prevented as the spore wall can be degraded. We prepared a half dilution of the pesticide as high concentrations can be hazardous to the human health. But, the pesticide still remains the best possible method and means to stop the insect and fungal pathogen and early spraying of this pesticide at the effective concentration of 43.5%w/w leads to the entire prevention of the spore germination. Hence, the mango production can be increased from its present status.

Acknowledgement

We want to express our greatest gratitude to the respected principal of our institution, St. Xavier's College, Rev. Fr. Dr. Felix Raj for providing the necessary assistance to carry out the work.

References

POPENOE W. (1974) Hafner Press, New York, USA, pp 79-145. Manual of tropical and subtropical fruits

Prakash Om , Diseases and Disorders of Mango and their Management (91) Department of Crop Protection, Central Institute for Sub-Tropical Horticulture. Rehmankhera, P.O. - Kakori, Lucknow, 227 107, India.

Harris K.M. and Schreiner I.H.,(1991) International Institute of Entomology, College of Agriculture and Life Sciences, University of Guam, A new species of gall midge (Diptera: Cecidomyiidae) attacking mango foliage in Guam, with observations on its pest status and biology

Sankaran T., Mjeni A.M. 2009, Recent studies on the mango leaf-gall midge *procontarinia matteiana* kieffer and cecconi (dip. cecidomyiidae) and its parasites in india and on prospects for biological control of the pest in oman , The University of Adelaide, School of Agriculture, Food and Wine, Australian Entomological Society

Insecticide fact sheet (2003) Journal Of Pesticide Reform• VOL. 23, NO.4

Sharma R.M. (2009), Checklist Of Indian Gall Midges (Diptera: Cecidomyiidae) Zoological Survey of India.

Mihai Aldea, Hamilton Jason G., Resti, Joseph P. Zangerl Arthur R., Berenbaum May R., Frank Thomas D., DeLucia Evan H. (2006) Compairison Of Photosynthetic Damage From Arthropod Herbivoryand

Pathogen Infection In Understory Hardwood Saplings.

Nelson Scot.C. (2008) Cercospora Leaf Spot and Berry Blotch of Coffee.

Windels Carol E., Plnt Pathologist, University of Minnesota, Northwest Research and Outreach Center, Bradley Carl A., Extension Plant Pathologist, North Dakota State University. Khan R Mohamed F.., Extension Sugar beet Specialist, North Dakota State University/University of Minnesota, Comparison Of Cercospora And Bacterial Leaf Spots On Sugarbeet.

Cincotta C. L., Adams J. M., Holzapfel C. (2008), Testing The Enemy Release Hypothesis: A Comparison Of Foliar Insect Herbivory Of The Exotic Norway Maple (*Acer platanoides* L.) And The Native Sugar Maple (*A. Saccharum* L.).

Rohfritsch Odette, Plants, gall midges, and fungi: a three-component system

Agrios,Plant Pathology

Duvenhage J.A. (2002), Evaluation Of New Generation Fungucudes For Control Of Cercospora Spot On Avocado Fruit, South African Avocado Growers' Yearbook(25: 11-14).

Brown Amy E., Mode Of Action Of Insecticides And Related Pest Control Chemicals For Production Agriculture, Ornamentals, And Turf, Pesticide Information Leaflet No. 43.

McCord Junior Elzie, Price James F. and Nagle Curtis A. (2002) Pesticide Mode Of Action Codes For Ornamental Growers In Developing Control Programs To Manage Pest Resistance.

Karima, Haggag H.E. and Sayeda, Farghaly F. (2007) Effect Of Metalaxyl And Chlorpyrifos-Methyl Against Early Blight (*Alternaria solani*, Sor.) And Whitefly (*Bemisia tabaci*, Genn.) In Tomato And Eggplant.