ACTIVITIES REPORT

OF

CHINESE DELEGATION VISIT

DEPARTMENT OF ENTOMOLOGY

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EXECUTIVE SUMMARY

Cotton is an important cash crop providing raw material for textile industry. It's share is 1.0 percent in country's GDP with 5.1 percent share in agriculture value addition. After introduction of Bt cotton, there was excellent control of bollworms like American bollworm, Spotted bollworm and pink bollworm. Pink bollworm almost was under check from 2004-2015 to 2014 on Bt cotton Bollguard-I expressing Cry1Ac. During fiscal year 2015-2016, production of cotton declined 27.8 percent as compared to last year 2014-2015 i.e. (10.074 million vs 13.960 million bales). Pink bollworm, *Pectinophora gossypiella* (Saunders) was identified at top among other possible causes of decline in cotton yield (Economic Survey of Pakistan 2015-2016, page # 24-26). Its population is increasing in the field from last three years 2015, 2016 and 2017 according to survey reports of Department of Pest Warning and Quality Control and Agriculture Extension Govt. of Punjab. For the detection of resistance, PBW should be reared on artificial diet and scientists need to be trained. Recently, Prof. Dr. Shafqat Saeed, Chairman Department of Entomology and Dean Faculty of Agriculture and Environmental Sciences, Muhammad Nawaz Shareef University of Agriculture Multan visited Institute of Plant Protection, Chinese Academy of Agricultural Sciences China. During his stay he signed MoU for training of scientists, experts, exchange of students related to Agriculture, for entomological aspects in particular Pink bollworm and whitefly.

A Chinese delegate of two scientists Dr. Wang Ling and Dr. Chengbo Cong from Hubei Academy of Agricultural Sciences, Wuhan, China visited MNS University of Agriculture Multan. They are experts of Pink bollworm rearing in the laboratory and resistance management experts. During their stay, they had meeting with faculty, students of Post graduation and Ph.D enrolled in Department of Entomology. They discussed different ongoing activities of research. There was exchange of views and knowledge among the researchers. An international seminar entitled "Pink bollworm Management: Chinese perspective" was organized by Department of Entomology, MNSUAM dated 21, May 2018. Mr. Zafaryab Haider, Director General Agriculture Extension & Adaptive Research Punjab attended as Chief Guest. More than 200 participants from Academia, Extension workers, Faculty, Students, Farmers and Representatives of private sector attended the seminar. Prof. Dr. Shafqat Saeed Dean, FAES delivered welcome address and purpose of Chinese delegation visit. Dr. M. Ishtiaq, Assistant Prof. Department of

Entomology talked on Pink Bollworm: Pakistan scenario. In his speech he provide the introduction of Pink bollworm, mode of damage, presence indicators, historical scenario of Pink bollworm outbreak, present situation of Pink bollworm in Punjab, reasons of outbreak and possible management options available for PBW. Ch. Asif Majeed, president Evyol Group Pvt. Ltd. talked about role of pesticides industry in Pink bollworm management. He told about different products available against pink bollworm. He highlighted the role of pesticide industry in farmer awareness, transfer of technology, Solution provision and serving as stakeholders in strategy development. Dr. Wang Ling Hubei Academy of Agricultural Sciences talked about management of Pink bollworm: Chinese perspective. She introduced her Institute and different research work being conducted at Hubei Academy of Agriculture Sciences China. She explained that they have developed an effective strategy of PBW management by sowing 25% refuge crop. They have well developed facility of PBW rearing and Bt resistance detection. She also welcomed Pakistani scientists to visit their lab and develop some joint projects. Mr. Zafaryab Haider, Director General Agric. Extension explained the role of Deptt. of Agriculture Extension Punjab. He also told that Govt. of Punjab provided subsidized cotton seed to the farmers at Union Council level in each tehsil. Prof. Dr. Asif Ali, Vice Chancellor, MNSUAM in his address appreciated the efforts of department of Entomology for organizing a wonderful event. He told that we are working with all stakeholders to address issues related to management of different insect pests. We shall find international collaborators, researchers working on different aspects of cotton in future as well. He also thanked all the participants' especially Chinese delegates for their visit to Pakistan.

Two days "International workshop for lab establishment and rearing techniques of pink bollworm" was organized at MNS-UAM on 22-23 May 2018. Dr. Wang Ling, Hubei Academy of Agricultural Sciences China was resource person. She provided training on preparation of artificial diet for pink bollworm, for laboratory rearing and requirements for establishment of laboratory. Practical demonstration of artificial diet preparation and shifting of first instar larvae on the diet. Requirements for controlled conditions of successful rearing of PBW in the laboratory. On second day of workshop, a lecture was delivered on bioassay techniques through diet incorporation method. She also share some results of their experiments conducted at China for resistance management through refuge strategy. They have managed PBW through 25% refuge crop. Almost 66 professionals were trained which includes faculty members, Scientists,

Researchers, post graduate and doctoral students of various universities like MNS-University of Agriculture Multan, University of Agriculture Faisalabad, Bahaudin Zakariya Univerty Multan, Layyah Campus Bahaudin Zakariya Univerty Multan, IUB, CEMB Punjab University Lahore, Peer Mehr Ali Shah Arid Agriculture University Rawalpindi scientists from various Research Institute i.e., Nuclear Institute of Biotechnology and Genetic Engineering, Ayub Agriculture Research Institute Faisalabad, Cotton Research Institute Multan, Central Cotton Research Institute, Multan, Maize and Millet Research Institute, Sahiwal. It will help capacity building of our scientists and opportunities for our scientists to develop contacts and get technical support in future. At the end certificates were distributed among the participants by Chief Guest, Vice Chancellor, MNSUAM.

Chinese delegation also visited cotton fields and rearing facilities established at Cotton Research Institute and Central Cotton Research Institute, Multan during their stay. Then they spent two days at Faisalabad and visited University of Agriculture Faisalabad, NIBGE Faisalabad, Ayub Agriculture Research Institute, Faisalabad and discussed different aspects of PBW research.

This visit was very successful in terms of learning, exchange of views, for developing international contacts of the field. It will help to share experiences among the scientist, researchers to develop joint research projects. Researchers also have an opportunity to develop links with Chinese and Pakistanis for getting technical, scientific equipments that were difficult to find out in the local market especially for Pink bollworm rearing materials and equipments. They were also excited to visit again in November to attend SINO-PAK International Cotton Conference to be held at MNSUAM this year. He also thanked Punjab Agriculture Research Board for providing funds for Chinese delegation visit to facilitate MNSUAM.

BACKGROUND

Cotton is an important cash crop providing raw material for textile industry. It's share is 1.0 percent in country's GDP with 5.1 percent share in agriculture value addition. After introduction of Bt cotton, there was excellent control of bollworms like American bollworm, Spotted bollworm and pink bollworm. Pink bollworm almost was under check from 2004-2015 to 2014 on Bt cotton Bollguard-I expressing Cry1Ac. During fiscal year 2015-2016, production of cotton declined 27.8 percent as compared to last year 2014-2015 i.e. (10.074 million vs 13.960 million bales). Pink bollworm, *Pectinophora gossypiella* (Saunders) was identified at top among other possible causes of decline in cotton yield (Economic Survey of Pakistan 2015-2016, page # 24-26). Its population is increasing in the field from last three years 2015, 2016 and 2017 according to survey reports of Department of Pest Warning and Quality Control and Agriculture Extension Govt. of Punjab. While situation is worst according to our recent survey in September-October 2017 in which we found pink bollworm infestation is more than it is reported in the fields, all Bt varieties were equally effected by this pest. Pink bollworm is the most destructive insect pest of cotton, caterpillars feed on the fruiting parts of the plant (flowers, squares and bolls) hence responsible for direct yield loss. It is found in almost all cotton growing areas of the world. Possible reason of recent outbreak is the development of resistance in Pink bollworm against Bt toxin i.e., Cry1Ac in Pakistan. If we see literature it has been reported worldwide from USA, China and India. All these countries adopted certain protocols to break resistance in pink bollworm. Unfortunately, there is not a single laboratory for resistance detection is available in Pakistan. For the detection of resistance, PBW should be reared on artificial diet and scientists need to be trained. Recently, Prof. Dr. Shafqat Saeed, Chairman Department of Entomology and Dean Faculty of Agriculture and Environmental Sciences, Muhammad Nawaz Shareef University of Agriculture Multan visited Institute of Plant Protection, Chinese Academy of Agricultural Sciences China. During his stay he signed MoU for training of scientists, experts, exchange of students related to Agriculture, for entomological aspects in particular Pink bollworm and whitefly.

VISIT OF LABS AND MEETING WITH FACULTY AND STUDENTS

A Chinese delegates of two scientists Dr. Wang Ling and Dr. Chengbo Cong from Hubei Academy of Agricultural Sciences, Wuhan, China_visited MNS University of Agriculture Multan. They are experts of Pink bollworm rearing in the laboratory and resistance management experts. During their stay, they had meeting with faculty, students of Post graduation and Ph.D enrolled in Department of Entomology. They discussed different on going activities of research. There was exchange of views and knowledge among the researchers.


Caption: Meeting with students and faculty of Entomology at MNSUAM


Caption: Prof. Dr. Shafqat Saeed briefing during lab visits

After that they visited MNSUAM-CRI Insecticides Resistance Monitoring Laboratory and Insect Rearing facility recently established by MNS University of Agriculture, Multan at Cotton Research Institute, Multan with collaboration of private sector. Dr. M. Ishtiaq, Assistant Professor, Department of Entomology MNSUAM briefed about different aspects and work being conducted. He also showed the management issues of different insect pests of cotton. They also

share some experiences of insect rearing aspects and issues of cotton crop. After that they visited cotton fields and check cotton crop.


Caption: Field and lab visit at Cotton Research Institute Multan

INTERNATIONAL SEMINAR ORGANIZED AT MNSUAM ENTITLED "PINK BOLLWORM MANAGEMENT: CHINESE PERSPECTIVE"

On the second day dated 21, May 2018, a seminar entitled "Pink bollworm Management: Chinese perspective" was organized by Department of Entomology, MNSUAM. Mr. Zafaryab Haider, Director General Agriculture Extension & Adaptive Research Punjab was invited as Chief Guest. Almost, 200 participants from Academia, Extension workers, Faculty, Students, Farmers and Representatives of private sector attended the seminar. Prof. Dr. Shafqat Saeed Dean, FAES welcomed the participants and guests. He briefly explained the importance of PBW and the purpose of visit of Chinese delegates. After that technical session started. First presenter was from Pakistan Dr. M. Ishtiaq Assistant Prof. Department of Entomology. He briefly explained Pink Bollworm: Pakistan scenario. In his speech he provide the introduction of Pink bollworm, mode of damage, presence indicators, historical scenario of Pink bollworm outbreak, present situation of Pink bollworm in Punjab, reasons of outbreak and possible management options available for PBW. He emphasized that we must adopt some advance techniques being adopted by other countries. He also shared experiences of different countries USA, China and India. He concluded that not a single control tactics is effective, we must adopt multiple options in integrated way and it is only possible with community involvement. All stakeholders i.e., farmers, PCGA, Agriculture department and private sector must play their role to combat this

pest. He also emphasized that continuity of policies adopted by Govt. of Punjab from last couple of years must be continued with same pace otherwise it will be wastage of resources.

Ch. Asif Majeed, president Evyol Group Pvt. Ltd. talked about role of pesticides industry in Pink bollworm management. He told about different products available against pink bollworm. He highlighted the role of pesticide industry in farmer awareness, transfer of technology, Solution provision and serving as stakeholders in strategy development. He also emphasized on integrated management of PBW through field sanitation, gin sanitation, use of pheromone and chemical control through synthetic pyrethroids.

Dr. Wang Ling Hubei Academy of Agricultural Sciences talked about management of Pink bollworm: Chinese perspective. She introduce about institute and different research work being conducted at Hubei Academy of Agriculture Sciences China. She explained that they have developed an effective strategy of PBW management by sowing 25% refuge crop. They have well developed facility of PBW rearing and Bt resistance detection. She showed some video related to laboratory rearing of some biological control agent of PBW. They have discovered parasitic wasps that is parasitoid of eggs of PBW that lay eggs inside the eggs of PBW and entered the body of larva of PBW. Infected larva then died and parasitic wasps emerged from it. She told that we are going to test this wasp in the field next year and if succeeded then they could share with Pakistan for its multiplication. She also welcomed Pakistani scientists to visit their lab and develop some joint projects.

Mr. Zafaryab Haider, Director General Agric. Extension explained the role of Deptt. of Agriculture Extension Punjab. He told that we implemented ban of cotton sowing before the month of April through section 144 in the whole Punjab a strategy given by researchers to reduce the damage of PBW on early sown cotton. He also told that we provided subsidized cotton seed to the farmers at Union Council level in each tehsil.

Prof. Dr. Asif Ali, Vice Chancellor, MNSUAM in his address appreciated the efforts of department of Entomology for organizing a wonderful event. He told that we are working with all stakeholders to address issues related to management of different insect pests. We shall find international collaborators, researchers working on different aspects of cotton. He also told that we are going to organize SINO-PAK International Cotton Conference in November, 2018 as we

did lat year in which different scientists will visit and present their research. He also emphasized that we must join hand for the progress of our famers which is the ultimate objective of all these institutions. He also thanked all the participants especially Chinese delegates for their visit to Pakistan.

ملتان۔محمد نواز شریف زرعی یونیورسٹی میں گلابی سنڈی کے خاتمے کے حوالے سے چائنیہ میں کئے گئے تجربات کی روشنی میں منعقدہ آگاہی سیمینار سے وائس چانسلر جامعہ پروفیسر ڈاکٹر آصف علی اور چائنیز سائنسدان سمیت دیگر خطاب کر رہے ہیں۔

TWO DAYS INTERNATIONAL WORKSHOP FOR LAB ESTABLISHMENT AND REARING TECHNIQUES OF PINK BOLLWORM ON 22-23 MAY 2018

Two days "International workshop for lab establishment and rearing techniques of pink bollworm" was organized at MNS-UAM on 22-23 May 2018. Dr. Wang Ling, Hubei Academy of Agricultural Sciences China was resource person. She provided training on preparation of artificial diet for pink bollworm, for laboratory rearing and requirements for establishment of laboratory. Practical demonstration of artificial diet preparation and shifting of first instar larvae on the diet. Requirements for controlled conditions of successful rearing of PBW in the laboratory. Prof. Dr. Asif Ali, Vice Chancellor MNSUAM welcomed all the participants and gave brief introduction of university. He told that participants that our university is progressing day by day. He briefed about construction work being carried out and ongoing research projects. He told that we have young dynamic faculty with full energy and enthusiasm. Prof. Dr. Shafqat Saeed, Dean Faculty of Agriculture & Environmental Sciences, MNSUAM introduced the Chinese delegates. Dr. Wang Ling and Dr. Shengbo Cong from Hubei Academy of Agricultural Sciences, Wuhan, China. Dr. Wang Ling gave briefing about Management Strategy and Artificial Rearing of Pink Bollworm. She gave brief introduction of their laboratory, some research progress of their team, artificial rearing of Pink bollworm.

She told that Institute of Plant Protection and Soil Fertility, Hubei Academy of Agricultural Sciences is located in Wuhan city, Hubei Province of China, which includes 73 researchers and workers at present. The research mainly involves the research and development of botanical pesticides, integrated control of disease, pests and weeds of main crops, safety evaluation of transgenic crops, monitoring and control of agricultural non-point source pollution, new fertilizer research. The main research objectives include pink bollworm, cotton leafworm (*Spodoptera litura*), vegetable whitefly, corn borer, parasitic wasps (*Chelonus pectinophorae Cushman*), cotton verticillium wilt. Integrated Resistance Management strategy for pink bollworm in China **(**Resistant individual can normally feed on Bt cotton). They have established resistant and susceptible strains of PBW in their laboratory at China. Individuals carried cadherin alleles could survive on Bt cotton. They found that Cadherin gene mutation is the main factor for resistance.

محمد نواز شریف زرعی یونیورسٹی ملتان میں کپاس کی فصل پر حملہ آور مہلک کیڑے گلابی سنڈی کے نقصانات کا اندازہ لگانے کیلئے لیبارٹری میں پالنے کے طریقے کے بارے میں منعقدہ دوروزہ انٹرنیشنل ورکشاپ سے وائس چانسلر جامعہ پروفیسر ڈاکٹر آصف علی، ڈاکٹر وانگ لنگ (Dr. Wang Ling) اور ڈاکٹر چونگ شینگبو (Dr. Cong Shangboa) خطاب کر رہے ہیں۔

On second day of workshop, a lecture was delivered on bioassay techniques through diet incorporation method. She also share some results of their experiments conducted at China for resistance management through refuge strategy. They have managed PBW through 25% refuge crop. Almost 66 professionals were trained which includes faculty members, Scientists, Researchers, post graduate and doctoral students of various universities like MNS-University of Agriculture Multan, University of Agriculture Faisalabad, Bahaudin Zakariya Univerty Multan, Layyah Campus Bahaudin Zakariya Univerty Multan, IUB, CEMB Punjab University Lahore, Peer Mehr Ali Shah Arid Agriculture University Rawalpindi scientists from various Research Institute i.e., Nuclear Institute of Biotechnology and Genetic Engineering, Ayub Agriculture Research Institute Faisalabad, Cotton Research Institute Multan, Central Cotton Research

Institute, Multan, Maize and Millet Research Institute, Sahiwal. It will help capacity building of our scientists and opportunities for our scientists to develop contacts and get technical support in future. At the end certificates were distributed among the participants by Chief Guest, Vice Chancellor, MNSUAM.

محمد نواز شریف زرعی یونیورسٹی ملتان میں منعقدہ دو روزہ انٹرنیشنل ورکشاپ کے پہلے روز HABEI اکیڈمی آف ایگریکلچر سائنسز Wuhan ووہان چائینہ کے زرعی سائنسدان لیبارٹری میں گلابی سنڈی کی مصنوعی خوراک تیار کرنے کا عملی مظاہرہ کر رہے ہیں۔

All of the resistant strains carried cadherin resistance allele.

Expression and location of PgCad47 in insect cell lines: The wild-type cadherin was mainly located on the cell membrane, whereas mutant cadherin were retained in the endoplasmic reticulum (ER), subcellular localization of these cadherin were incorrect.

Natural Enemy of Pink Bollworm: Dr. Ling Wang told about the Natural enemy in pink bollworm, main natural enemies of pink bollworm were a braconid wasp (*Chelonus pectinophorae Cushman*) and an ichneumon wasp (*Pristomerus chinensis Ashmead*). *Chelonus pectinophorae Cushman* is the egg parasitoids, while *Pristomerus chinensis Ashmead* is the larval parasitoids.

She told that parasitic wasp was recovered from overwintering generation of pink bollworm in 2013, and it was reared in the lab successfully at temperature of 28±1℃, RH 60-80%, L:D= 14:10. Adults need to supply for 8% honey water to provide extra-nutrition.

The *Chelonus pectinophorae* adults were active during 5:00-19:00 o'clock. The courting and mating behavior between male and female adults mainly occurred during 7:00-9 :00 o'clock. Female adults laid eggs among 7:00-17:00 o'clock.

There were three parasitic behaviors of *C. pectinophorae* including searching, oviposition and cleaning. First, wasp need to search and identify the eggs of pink bollworm using their antenna, then the female insert their ovipositor into the eggs. A single female can lay eggs continuously for 25 to 70 times, and it takes average 20 seconds each time. During spawning, the female will clean the antenna using their fore legs.

After 8 days, the larvae of wasp become mature, then the larva drilled out from a side of prothorax of the pink bollworm, and started eating the host's body until there was mere head shell, next step is preparing for spinning a cocoon. After being infected by *Chelonus pectinophorae*, the body length and weight of 2-4 instar pink bollworm larvae were significantly lower than those of control group.

Artificial Rearing of Pink Bollworm

Dr. Ling Wang briefed that there are more than ten strains.

- Strains from China: susceptible strain (CS) and resistant strains (JL-25, JL-46, AQ-47, AQ-65, AQ-189, QJ-62, TM-6)
- Strains from America: susceptible strain (APHIS-S) and resistant strains(AZP-R)

Instruments: analytical balance, oven, pulverizer, induction cooker, blender, clean bench.

Raw materials and reagents: wheat germ, casein, sucrose, yeast powder, cellulose, potassium sorbate, Nipagin, calcium pantothenate, Niacin, folic acid, riboflavin, thiamine (B1), pyridoxine hydrochloride (B6), vitamin B12, biotin, choline chloride, agar, corn oil, honey, 19 species

Baking and pulverizing wheat germ:

- Put the wheat germ in the porcelain dish, and put it in the oven, bake for 8 hours at 85℃
- Turn the wheat germ into powder with pulverizer, then keep in reserve at 4 ℃

The ingredients are weighed accurately with an electronic balance according to the formula:

Artificial diet ingredients for PBW rearing	
Name of ingredient	**Weight (g) or Vol (ml)**
Wheat germ	34.5
Casein	30
Agar	24
Sucrose	10
Yeast powder	5
Cellulose	1
Potassium sorbate	1.5
Nipagin	0.5
Decavitamin	0.01
Choline chloride	0.06
Corn oil	3.3
Honey	2
Water	730

Components of multivitamins	
Name	**Weight (g) or Vol (ml)**
Calcium pantothenate	12
Niacin	6
Riboflavin	3
Folic acid	3
Thiamine (B1)	1.5
Pyridoxine hydrochloride (B6)	1.5
Biotin	0.93
Vitamin B12	0.012

Preparation of Artificial diet:

Agar strip is added in water to heat it to boiling until agar is completely dissolved; Mixed the mixture well with the dissolved Agar in a blender, then pouring into a dish after fully mixed.

After diet is cooled and solidified, the diet is shredded into small pieces and dispensed into 24-well culture plates, then fan for 15 min.

Rearing management of pink bollworm:

Insects inoculated: First instars larvae are then transferred to artificial diet of 24-well culture plates, each well inoculated with one or two larvae, then put lid on the plate immediately after inoculating to prevent the larvae from escaping.

Management of diet: After inoculating, the culture plates will be put in an insectary of 28±1℃, 40±10% RH. It will take about 8 days for the larvae to reach 4th instar, and approximately 12 days the larvae begin to pupate. Air conditioning and dehumidifiers are regularly checked during this period to ensure the constant temperature and humidity in the insectary.

After pupation, pupae will be collected from culture plates and put in a plastic to shift them in glass cages for emergence in an insectary of 28±1℃, 70±10% RH. Adults will be provided with 8% honey solution, and honey water will be changed every two days. Collect spawning paper each day and put it into a glass bottle. Eggs will hatch into larvae for about 3-4 days at 28±1℃, 70±10% RH.

Common Problem: The major problems**:**

1. Black fungus and bacteria on the diet.

2. Pink bollworm larvae parasitized by the parasitic mites.

Solutions to the above problems:

1) All of the 24-hole cell culture plates and cages for adults laying eggs used each time were treated with 84 disinfectant (main ingredients: sodium hypochlorite) for 24-48 hours

2) The clean bench used for placing artificial diet should be sterilized for 20 minutes with ultraviolet lamp every day.

3) Each insectary is regularly treated with miticide, high temperature and ultraviolet irradiation, and equipped with an anti-mite meter.

4) The pink bollworm collected from the field should be separated from the pink bollworm in the lab, because the individuals from the field may carry some parasitic mites, if place them together, it could lead to extinction of the entire population.

All the participants were provided with manual containing information regarding artificial rearing of pink bollworm and culture handling, prints of presentations of experts.

Before returned to China they spent two days at Faisalabad and visited University of Agriculture Faisalabad, NIBGE Faisalabad, Ayub Agriculture Research Institute, Faisalabad and discussed different aspects of PBW research. This visit was very successful in terms of learning, exchange of views, for developing international contacts of the field. It will help to share experiences among the scientist, researchers to develop joint research projects. Researchers also have an opportunity to develop links with Chinese and Pakistanis for getting technical, scientific equipments that were difficult to find out in the local market especially for Pink bollworm rearing materials and equipments. They were also excited to visit again in November to attend SINO-PAK International Cotton Conference to be held at MNSUAM this year.