Food and Agriculture Organization
of the United Nations

Training Workshop: "Management of Aquatic Weed - Water Hyacinth, *Eichhornia crassipes*"

Mansoura, Egypt
25 - 27 August 2015

PROCEEDINGS

Training Workshop: "Management of Aquatic Weed - Water Hyacinth, *Eichhornia crassipes*"

Mansoura, Egypt 25-27 August 2015

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Chapter 1

The report of the training workshop:
Management of Aquatic Weed – Water Hyacinth, *Eichhornia crassipes*

Introduction

Water hyacinth, the world's worst aquatic weed, causes annual losses to irrigation schemes, fisheries, riparian communities, hydro-electricity generation and water transport in excess of US$100 million. Resource-poor communities that rely on lakes and rivers for their survival are severely affected by this noxious weed due to reduced access to water, reduced water quality, increased health problems from bilharzia, malaria, cholera and snakes, and a reduction in household income through a decline in fishing activities.

The workshop was a cooperation between the Food and Agriculture Organization of the United Nations (FAO) Regional Office for the Near East and North Africa (NENA) and the Faculty of Agriculture of Mansoura University.
The workshop took place at the Faculty of Agriculture of Mansoura University during 25-27 August 2015. Nineteen participants from five Nile Basin countries participated in the workshop (Egypt, Sudan, Ethiopia, Kenya and Tanzania) in addition to the FAO Regional Crop Protection Officer for the NENA Region and the FAO-AGP Weed Management Officer.
The objectives of this training workshop were:

- to understand the current situation of water hyacinth in the Nile basin countries and discuss the challenges facing the sound management of the weed and the programmes and strategies used for its management in each country;
- to exchange experiences and knowledge about the management of water hyacinth among the participating countries of the Nile Basin;
- to increase knowledge and practical skills around innovative methods for the biological control of water hyacinth and its applications, using safe, non- polluting and cost-effective bioherbicides;
- to enhance collaboration among specialists and related institutions in the Nile basin countries for implementing bioherbicidal control methods for sustainable water hyacinth management;
- to prepare the necessary recommendations to overcome the obstacles and challenges faced by participating countries in the implementation of management programmes for water hyacinth;
- to discuss the possibility of proposing a regional project or a common framework to assist participating countries in facing these challenges.

- First day: Tuesday, 25 August 2015

- **Opening Session: 11.30 a.m. – 12.15 p.m.**

The opening session started at 11.30 a.m. in the Grand Conference Hall, Faculty of Agriculture with a welcome speech by Dr. Yasser Shabana, the Vice Dean for Graduate Studies, Research and Cultural Affairs and the Workshop Rapporteur.

This was followed by a welcome speech by Dr. Yasser El-Hadidi, Dean of the Faculty of Agriculture. He briefly spoke about the importance of the workshop topic and of the decision by FAO and the Faculty of Agriculture of Mansoura University to address this topic in a cooperative regional training workshop. Next, Dr. Shoki Al- Dobai, Crop Protection Officer, FAO Regional Office for the Near East (RNE) delivered extended lengthy speech. He welcomed the audience of international and national participants and stressed the valuable collaboration between the FAO and the Faculty of Agriculture, Mansoura University on a critical issue for the environment and food production and a common challenge for all participating countries: the management of water hyacinth. Dr. Al- Dobai spoke about the framework priorities and five strategic objectives of the FAO. He presented the activities of the FAO Department of Plant Production and Plant Protection. He also briefly spoke about the importance of weeds, the challenge they pose to farmers and the environment in general, and the three pillars that FAO's strategy uses to promote integrated weed management. Then he talked about the problems and damage caused by water hyacinth and that FAO regards this workshop as seeking means to – through collaboration with research and academic bodies – provide technical support and exchange of experiences and knowledge to help combat this troublesome aquatic weed. Finally, he described the objectives of the workshop, as stated in the Introductory section above.

The opening session was concluded by a speech from Drr. Magda Nasr, the Vice President of Mansoura University, who welcomed international and national participants and guests and expressed appreciation for the collaboration with FAO in launching this training workshop at the Faculty of Agriculture of Mansoura University. She stressed the great importance of the workshop topic, not only for Egypt but also for the Nile basin countries and worldwide. She also highlighted the profile of Mansoura University among national and African universities.

The opening session and the rest of the workshop activities were delivered in Arabic and accompanied by English interpretation for non-Arabic speakers using an instant translation system.

- **First Session: 12.30 p.m. – 2.10 p.m.**

Chaired by: **Yahia Fayad**, Plant Protection Research Institute, Agricultural Research Center, Giza, Egypt.

This session contained two key-note presentations, as follows:

- **Lecture 1. Can biological control really work in diverse weed systems?**
  Yasser Shabana, Faculty of Agriculture, Mansoura University, Egypt

In the first keynote lecture, Yasser Shabana presented the results of his research on the biological control of four weed systems: water hyacinth (*Eichhornia crassipes* or floating aquatic weed); hydrilla (*Hydrilla verticillata* or submerged aquatic weed); sunflower broomrape (*Orobanche cumana* or root parasitic weed) and common waterhemp (*Amaranthus tuberculatus* or crop weed). The research was carried out at Mansoura University, Egypt; the University of Florida, USA; University of Hohenheim, Germany; and Purdue University, USA. He focused on the water hyacinth system and presented his research results dating back over 30 years to 1983. A strain of *Alternaria eichhorniae* (Ae5), isolated from water hyacinth in the Nile Delta, has been shown to be an effective bioherbicide candidate for water hyacinth. In an attempt to develop this bioherbicide, various formulations of the fungus, its sporulation, phytotoxin production and impact on water hyacinth growth were studied. The optimum conditions for the production of highly virulent inoculum and the epidemiological requirements for disease incidence and severity were also investigated. The physiological and ultrastructural host responses were explored as well to furnish background information for understanding the host- pathogen pathosystem. Results from laboratory, greenhouse and preliminary field trials confirmed the feasibility of using the fungus to control water hyacinth. However, a major obstacle to the use of Ae5, as a foliar pathogen for water hyacinth is the need for at least ten hours of dew to enable the fungal propagules to germinate and infect and, to an extent, to colonize the weed. Such extended exposure to dew periods is not likely to occur under field conditions. For this reason, several approaches to overcoming the lack of dew under the field conditions have been explored, e.g. formulating the fungal inoculum in hydrophilic polymers, in invert emulsions and in vegetable oil suspension emulsions. The latter two formulations were successful in that they averted dew dependence in greenhouse trials. The cottonseed oil formulation produced 100% water hyacinth control, seven weeks after treatment. This and other innovative formulations must be tested under field conditions and the practical utilization of this fungus as a bioherbicide must be undertaken as a realistic goal for cost-effective, non-polluting and environmentally-friendly solutions to the water hyacinth problem.

- **Lecture 2. Continent-wide strategic framework on management of invasive alien plants in Africa.**

  Gualber Gbehounou, FAO Weed Management Officer, Rome, Italy

In the second keynote lecture, Gbehounou focused on invasive alien plants, their negative impacts and why a continental strategic framework is needed to defeat them. He considered a process for developing a continent-wide strategic framework, including participants in the process, the five pillars and operational structure. He presented examples of the invasive alien plants that pose severe problems for Africa, such as water hyacinth, the fern *Salvinia molesta, Parthenium hysterophorus* (a new threat in East Africa), and *Solanum elaeagnifolium* (a threat to agricultural production in Northern and Southern Africa).

The current workshop on use of bioherbicide to control water hyacinth fits under pillar three of the Continent-Wide Strategic framework: “Research and capacity development for IAP prevention and management”.

- **Second Session (Country Presentations): 3.20 p.m. – 4.30 p.m.**

Chaired by: **Gualber Gbehounou,** FAO Weed Management Officer, Rome, Italy.

This session contained two keynote presentations, as follows:

- **Update Status of Water Hyacinth Management in Egypt**
  Yahia Fayad, Plant Protection Research Institute, Agricultural Research Center, Giza, Egypt

As in many parts of the world, water hyacinth creates serious problems for the environment in Egypt. The weed plant not only affects water quality, use, flow, irrigation, navigation and fishing, it also poses health risks by enabling the breeding of mosquitoes, bilharzia and other human parasites. Thick mats of water hyacinth cover water bodies, reducing light and oxygen, drastically affecting water chemistry and aquatic life and greatly increasing the rate of evaporation of water. In Egypt, the total length of water bodies is 175 000 km, of which 116 000 km has been infested with water hyacinth (estimated to be 638 km^2) covering 66% of Egypt’s lakes, drainage and irrigation canals. The total amount of water loss by evapotranspiration from this infested area is estimated to be 3 million m^3 per day (1.1 billion m^3 per year). This amount is sufficient to irrigate 432 km^2 every year. This issue became more significant in view of the fact that the demand for irrigation water is increasing as a consequence of newly reclaimed areas for agriculture in Egypt. Water hyacinth is also a major threat to the ecosystem and biodiversity, affecting fish and aquatic faunas, plant community structure and diversity, water supplies and human health. It has also major impacts on multiple economic activities and community livelihoods.

The Egyptian authorities have banned the use of chemical herbicides in all water bodies since 1990, due to environmental and health concerns. Water hyacinth has typically been controlled through mechanical or manual removal, which costs more than US$ 8.5 million every year (Egyptian Minister of Irrigation, 2014). Physical removal does not provide any significant reduction of weed stands and water hyacinth continues to be a serious problem.

The presentation focused on using the *Neochetina eichhorniae* and *Neochetina bruchi* weevils for the biocontrol of water hyacinth in four natural lakes in Egypt (Al-Manzalah, Mariout, Edko and Al-Borollos) since 2000. The weevils were imported from Fort Lauderdale, Florida in 2000 under a five-year project "Water Hyacinth Biological Control" supported by France. The weevils have been subjected to quarantine procedures, pest risk analysis and host specificity tests. The presentation reviewed the weevils' rearing facility/greenhouse structure, requirements and methodology. Using the weevils for biological control has significantly reduced the water hyacinth cover of the treated lakes. The impact of the project was monitored and mapped through a remote sensing technology/satellite imaging.

Based on 2008 satellite images, the infestation of the water hyacinth has been reduced by around 96%, 65%, 80% and 96% in Edko, Mariout, Al-Manzalah and Al- Borollos Lakes, respectively. Using the biological control dramatically reduced the cost of water hyacinth action, amounting to around 0.5% of the cost of the mechanical and other traditional methods of control. The biological control requires around US$ 500 000 annually to maintain the continuity of production and release of the natural enemies, while, the annual budget allocated for mechanical control is around US$ 8.5 million.

- **Update status of water hyacinth management in Sudan**
  Eshraga Hussien, Plant Protection Directorate, Khartum, Sudan

Water hyacinth was declared a national pest in Sudan in 1959. It is causing a vast amount of water loss (about 7.1 billion cubic metres annually). This loss is almost double the amount that can be gained from the construction of Jonglei Canal (about 4 billion cubic metres). This lost amount can secure the irrigation of over 2 000 feddans of various crops and provide much needed water to Egypt. Water hyacinth also threatens the livelihoods of millions of people along the Nile Basin. It has negative effects on biodiversity, agricultural production, livestock, fisheries and human health. Today, the water hyacinth problem is growing in severity and geographic extent. Conventional control strategies of chemical, mechanical and legislative measures are costly and unsuccessful. Currently, Sudan has a collaborative project with Egypt to use the *Neochetina eichhorniae* and *Neochetina bruchi* weevils for the biocontrol of water hyacinth in Sudan and there is interest to integrate that with bioherbicides.

- Second Day: Wednesday, 26 August 2015

▪ First Session (Country Presentations - Continued): 08.30 a.m. – 10.00 a.m.

Chaired by: **Shoki Al-Dobai**, Crop Protection Officer, FAO Regional Office for Near East (RNE), Cairo

This session contained three keynote presentations, as follows:

➢ **Update status of water hyacinth management in Ethiopia**
Wassie Melkamu, Bahir Dar University, Bahir Dar, Ethiopia

Foreign experts employed at the dam site introduced water hyacinth into Ethiopia (at Aba Samuel Dam) as an ornamental plant in 1950s. Its occurrence in Koka Lake (Awash River) was reported in 1965. It then spread to Baro-Akobo Rivers (Gambella), and more recently (2011) to Lake Tana and other bodies of water. Tana is the largest lake in Ethiopia at 32 000 km^2, and one of the 250 lakes selected as global biodiversity conservation region by Lakenet since it is home to 15 unique Labeobarbus fish species. In 2011, water hyacinth infestations covered 4 000 ha of Lake Tana, increasing in 2012 to cover 20 000 ha of the north eastern shore and 40 km of shoreline over three districts (Libokemkem, Gondar Zuria and Dembiya) and 15 kebeles. In August 2014, infestation by water hyacinth in Lake Tana reached up to 50 000 ha in five woredas and along 128 km of shoreline. A survey conducted in May 2015 showed some reduction of the infested area in Lake Tana to 34 500 ha due to physical removal and the dormancy of the weed during the dry period.

Water hyacinth has been effectively controlled in 116 ha of water reservoirs and 15 km of primary and secondary canals of Wonji/Shewa sugar plantations by draining all the water reservoirs and burning the accumulated dried herbage on the reservoir floors after glyphosate application. The seed bank in the top layer of the soil was removed using heavy machinery. Refilled reservoirs are being monitored to ensure that no re-infestation occurs.

In June 2012, the regional government decided to remove the weed with the active participation of the community. The Regional Water Hyacinth Steering Committee organized subcommittees at zonal, woreda, and kebele levels and, in October 2012, a voluntary public campaign was organized in all water hyacinth-infested kebeles. A total of 212 779 work days (193 230 male and 19 549 female) was expended for the manual removal of the weed. As of May 2015, 23 487 ha of water hyacinth (92% of the weed-infested area) in Lake Tana had been manually removed and burned after drying. However, challenges remain, including declining motivation of local people, poor international and national support, lack of expertise in using other control methods, poor infrastructure (road and landing) and the lack of boats, vehicles, etc.

The release of insect biocontrol agents is also being considered as an integral component of an approach to mechanical and chemical control. The Ethiopian Institute of Agricultural Research (EIAR) and the Ethiopian Sugar Corporation Research Directorate at Wonji introduced two weevils *(Neochetina eichhorniae and N. bruchi*) from Uganda through a United Nations Environmental Programme (UNEP) / Global Environment Facility (GEF) Removing Barriers to Invasive Plant Management in Africa (RBIPMA) Project. The weevils are currently in the mass rearing stage in lath-houses at Wonji and it is proposed to release these biocontrol agents in the neighboring localities of the Koka Lake area once approval has been received from the Federal Environmental Protection Authority. The pre-release and release plans have not been implemented yet due to lack of funding.

Nineteen indigenous fungal species were found to be associated with water hyacinth. The nine most virulent species were selected based on preliminary tests. Host range tests of these potential biocontrol agents were conducted with economically important plants in the Ethiopian Rift Valley. Following an efficacy test under field conditions, it was found that *Altenaria altenata* and *Pythium ultimum* could be used for water hyacinth biocontrol together with insects.

- **Update Status of Water Hyacinth Management in Kenya**
  Reuben Omondi, Kisii University, Kisii, Kenya

Water hyacinth was first sighted in the Kenyan waters of Lake Victoria in June 1991. The weed then invaded small water bodies in the basin. By 1998, the weed covered an area of approximately 17 231 ha, mostly in the Nyanza Gulf. *Neochetina* weevils were imported in 1997, mass reared and released into the lake. The coverage by December 1999 was 3 100 ha, which was further reduced to 530 ha by February 2000. In 2000, mechanical harvesters were used to control the weed in Kisumu Bay. Since then, there have been increases and decreases in the acreage covered by the weed in the lake. Decreases have been attributed to biological control agents and macrophyte succession.

From 1997 to 2002, a water hyacinth management project in Lake Victoria (LVEMP I) was funded by the International Development Association (IDA) and the Global Environmental Facility (GEF) at a total budget of US$70 million. The objective was to control water hyacinth infestation to enable the rehabilitation of the lake's ecosystem for the benefit of the 30 million people who live in the catchment. The project achieved successful water hyacinth control through training, the importation of biological agents and successful multiplication and inoculation.

The World Bank – through IDA – funded the second phase of the Lake Victoria Environmental Management Project (LVEMP 11) from 2009 to 2017. The project's US$30 million budget was intended to support the revival of weevil multiplication centers, the procurement of mechanical harvesters for use in hotspot areas, the establishment of a national water hyacinth surveillance and monitoring unit, training for communities on the surveillance and monitoring of water hyacinth and biological control methods, and periodic surveillance and monitoring of water hyacinth and other aquatic plants. However, weevils have not yet been introduced into the lake under this project, theharvester has not been procured, and the national water hyacinth surveillance and monitoring unit has not yet been established.

In Winam Gulf, water hyacinth covered 4 000 ha in December 2014 and by the end of July 2015, the infested area was 7 000 ha. Between those dates, the area of water infested with hyacinth underwent significant fluctuation.

- **Update status on water hyacinth management in Tanzania**
  Msami Elibariki, Ministry of Agriculture, Kibaha, Tanzania

Water hyacinth was first observed in Tanzania in 1955 in the Sigi RIver and in the Pangani River in 1959. By 1987, the weed had spread to the Kagera RIver, which flows into Lake Victoria. In 1988, water hyacinth was first observed in Lake Victoria. The lake has a total surface area of 68 800 km2 and a shoreline of about 3450 km, of which 33% is in Tanzania, which shares about 51% of the water of the lake with Kenya. By 1995, about 700 ha of water had been covered by the weed. In 1998, the coverage in Lake Victoria was estimated at 2000 ha (LVEMP report, 2000).

Water hyacinth has negative impacts on fish production, fishing operations, commercial transportation services on the lake and water intakes at the hydroelectric power station in Pangani river. It provides breeding habitats for the alternative host for schistosomiasis (also known as bilharzia) and increases the role of mosquitoes as a vector for malaria.

Water hyacinth is managed through the use of specialized machines, manual removal, chemical herbicides (Glyphosate and 2,4-D) and biological control agents: the weevils *Neochetina eichhorniae and N. bruchi*. A survey conducted in 2004 indicated that overall weed reduction had reached 80%. However, a survey conducted in 2007 revealed an increase in weed cover in lake bays and shores. This may be attributed to the flushing of water hyacinth from the Kagera River, which originates from Rwanda and drains into the lake (0.2-0.8 ha/day). In 1997, LVEMP Phase-I, funded by World Bank, was initiated. The project carried out the task of mass rearing weevils and releasing them in main infested areas on Lake Victoria, while 14 sub-rearing units were established along the lake on the Tanzania side. However, Rwanda and Burundi were not involved in LVEMP Phase- I.Thus,adequate control measures to reduce the weed influx from the Kagera River were unavailable and the status of water hyacinth infestation in the inland waters of Rwanda and Burundi has not been clearly documented.

- **Second session (practical training): 10.30 a.m. - 1.30 p.m.**

Chaired by: **Shoki Al-Dobai**, Crop Protection Officer, FAO Regional Office for Near East (RNE), Cairo

This session contained one lecture and one practical training, as follows:

➢ **Lecture. Biological control of weeds by using plant pathogens**
Yasser Shabana, Faculty of Agriculture, Mansoura University

Basic information about the biological control of weeds using plant pathogens was presented, including definitions of biological control and biocontrol agents; pros and cons of biological control; a comparison between chemical pesticides, biopesticides and bioherbicides; and formulation of microbial biocontrol agents and types of formulations, etc.

- **Practical session. Biological control of weeds by using plant pathogens**
Yasser Shabana, Faculty of Agriculture, Mansoura University

The Pictorial Manual on the Use of *Alternaria eichhorniae* as a Mycoherbicide for Water Hyacinth (Shabana, 2015; 2nd Edition) was handed to all participants. The objective of the session was to demonstrate themes of isolation, identification, pathogenicity testing, host range testing, culturing and mass production, spore production, phytotoxin production, and the application of *A. eichhorniae* as the most promising mycoherbicidal agent for water hyacinth in Africa. Participants also practiced scoring disease severity using a pictorial disease scale.

- **Third Session (Conclusion): 1.45 p.m. – 3.30 p.m.**

Chaired by: **Wassie Melkamu**, Bahir Dar University, Bahir Dar, Ethiopia; and Reuben Omondi, Kisii University, Kisii, Kenya

In this session, conclusions, recommendations and follow up actions, evaluation of the training workshop were discussed. This was followed by closing of the training workshop

General findings of the workshop

- Water hyacinth is still a problem in most countries because of its impact on water quantity and quality, biodiversity, transport etc.
- Water hyacinth management programmes in most country are mainly based on biological and manual/mechanical control methods.
- Research on the use of bioherbicides in Egypt is very promising.
- Biological control using *Neochetina* weevils has been successful in controlling water hyacinth in some countries.

- There is no/insufficient national coordination on the management of water hyacinth.
- Communities are involved in water hyacinth management in some countries.
- Mechanical and manual control of water hyacinth is critical for small water systems and canals.
- Egypt and Sudan are cooperating on the management of water hyacinth.
- The lack of cooperation between neighboring countries could weaken management results.

Opportunities for improvement

- There is commitment at the international level for management of aquatic and terrestrial IAS. Government should take advantages of these opportunities.
- Availability of indigenous bio-agents in the countries that need further efforts to use them in integrated management.
- Cooperation between different countries for joint management of water hyacinth.
- Community mobilization.
- There is room to expand cooperation between the Nile Basin countries.

Follow up action/ Recommendations at national level

- The government should recognize the pronounced research work done on *Alternaria eichhorniae* bioherbicide in the last 25 years at Mansoura University, support and facilitate its validation in the field.
- Need for surveillance and regular monitoring of the weed in different aquatic systems by using most appropriate technology.
- Establishment of national coordination units for management of Water hyacinth to ensure synergy among stakeholders.
- Governments are advised to incorporate Water hyacinth management in their policies and to identify sustainable funding mechanisms to sustain management programmes.
- Governments should make effort to mobilize additional resources to scale up management programmes.

Recommendations for strengthening cooperation/efforts at regional/ interregional levels

- FAO supports the formulation of an interregional project proposal on integrated management of water hyacinth and other invasive alien aquatic plants in Africa.
- Countries should support the formulation of an interregional project and identification of potential donors interested in biodiversity and climate change, such as the Global Environmental Facility (GEF) and the Green Climate Fund (GCF).
- Participants are encouraged to establish aquatic weeds information networks to share knowledge and information among themselves and with others.

- Third Day: Thursday, 27 August 2015

Field Trip to Sites of Water Hyacinth Infestations

All participants visited nearby sites of water hyacinth infestation. It was noticed that the fungal biocontrol agent, *Alternaria eichhorniae*, and the biocontrol weevils are coexisting in the same areas **(Figures 1 and 2).**

Figure 1. *Alternaria eichhorniae* disease symptoms as well as the feed spots of the biocontrol weevils, *Neochetina* spp., on water hyacinth plants.

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Figure 2. Yasser Shabana holds a leaf showing *Alternaria eichhorniae* disease symptoms and Yahia Fayad looks for weevil larvae in a leaf petiole.

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Annex 1: Workshop programme

Tuesday, 25 August 2015 (Grand Conference Hall, Faculty of Agriculture)	
11.00 - 11.30	Registration of participants
11.30 - 12.15	Welcome and Opening Ceremony - Speech by the FAO Representative - Speech by the Dean of Faculty of Agriculture - Speech by the Vice President of Mansoura University
12.15 - 12.30	**Coffee Break**
12.30 - 1.30	**Lecture – Can biological control really work in diverse weed systems?** Yasser Shabana, Faculty of Agriculture, Mansoura University
1.30 - 2.10	**Continent-Wide Strategic Framework on Management of Invasive Alien Plants in Africa -** Gualber Gbehounou, FAO Weed Management Officer, Rome, Italy
2.10 - 3.20	**Lunch**
3.20 - 4.00	**Presentation on Update Status of Water Hyacinth Management in Egypt** Yahia Fayad, Plant Protection Research Institute, Agric. Research Center, Cairo
4.00 - 4.30	**Presentation on Update Status of Water Hyacinth Management in Sudan** Eshraga Hussien, Plant Protection Directorate, Khartum, Sudan
4.30 - 5.00	**Presentation on Update Status of Water Hyacinth Management in Ethiopia** Wassie Melkamu, Bahir Dar University, Bahir Dar, Ethiopia
5.00 - 5.30	**Presentation on update status of water hyacinth management in Kenya** **Reuben Omondi, Kisii University, Kisii, Kenya**
8.00 - 9.30	**Welcoming Dinner**
Wednesday, 26 August 2015 (Seed and Tissue Pathology Lab)	
08.30 - 09.00	**Presentation on Update Status of Water Hyacinth Management in Tanzania** Msami Elibariki, Ministry of Agriculture, Kibaha, Tanzani
09.00 - 10.30	**Lecture –Biological Control of Weeds by Using Plant Pathogens** Yasser Shabana, Faculty of Agriculture, Mansoura University
10.30 - 11.00	**Coffee Break + Group Photo**
11.00 - 1.30	**Practical Session:** Yasser Shabana - Isolation of Weed Pathogens - Pathogenicity Testing of Microbes Obtained against Water Hyacinth - Mass Production of the Biocontrol Agent – Spore Production - Bioherbicidal Formulation Techniques - Evaluation of Disease Severity Using a Disease Scale
1.30 - 1.45	**Coffee Break**
13.45 - 3.30	Evaluation, Discussion of the Recommendations and Follow up Actions, and Closing of the Training Workshop
3.30 - 5.00	**Lunch**
Thursday, 27 August 2015	
09.00 - 1.00	Field Trip to Sites of Water Hyacinth Infestations
1.00 - 2.30	**Lunch**
3.00	Departure of the participants from Mansoura

Annex 2: List of participants

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Annex 3: Results of the workshop evaluation

1. How do you evaluate the quality of the workshop in general?

- Content	Good	12
	Average	0
	Unsatisfactory	0
- Materials presented/handouts	Good	9
	Average	1
	Unsatisfactory	0
- Overall organization of the workshop	Good	8
	Average	3
	Unsatisfactory	0

2. Allocation of time

	Good	10
	More time for Discussion	0
	More time for Presentations	0

3. Duration of the meeting

	Too short	0
	About right	12
	Too long	0

4. Do you consider the topics discussed useful for future work?

	Very useful	10
	Partly Useful	2
	Not useful	0

5. How did the trainer handle his role?

	Good	10
	Okay	2
	Poor	0

6. How suitable, accessible and comfortable was the meetin gvenue?

	Good	7
	Okay	5
	Poor	0

7. Did the workshop meet your expectations?

	Yes	10
	To an extent	2
	No	0

8. Suggestions for improvement

- Use of pathogen in other water hyacinth-affected countries.
- Need for collaborative project proposals to control water hyacinth in all affected countries in Africa.
- All the countries must control water hyacinth at the same time using the most appropriate methods of control.

- Most of the presentations or discussions were on fungal pathology usage as bio-control for Water hyacinth but if weevil rearing techniques and associated necessary facilities were discussed, that will be a balance/or makes the training on biological control more complete.

9. Additional recommendations

- Organizers should meet and assist at the airport and provide transportation to the hotel.
- More services at the hotel in Mansoura.
- Pay for services and increase the daily miscellaneous expenses to be suitable to the needs.
- Inclusion of pathogen for control of water hyacinth is very important especially in hot spot areas.
- Request for application of pathogen in other countries.
- Communicate with Nile Basin countries in order to establish a comprehensive project for management of water hyacinth.
- Plan for a programmed/periodical environmental survey by FAO, the reports to be shared among countries.
- Increase capacity building among participating countries.
- Request presentations to be sent to participants by e-mail.
- More practical training sessions needed for fungus isolation and application.
- More specialized researchers in water hyacinth biological control in African countries -other than Egypt- should be participate in this very beneficial training. Would ask for future participation from plant ecologists and experts in plant population dynamics from different African countries.
- It was apparent that other areas were not presented in the workshop and that more participants should have been invited. During the workshop, it became apparent that it is necessary to combat invasive alien species on the continent. It is important that a project should be established to follow this.
- This interregional training on widely distributed weeds, such as water hyacinth, is a good strategy to take work on in a synchronized way rather than country-by-country or by focusing on regions independently.
- The biological control of water hyacinth needs more efforts for integrated control by different methods covering all countries in the Nile Basin.

Annex 4: Presentations

1. Can biological control really work in diverse weed systems?
   Yasser Shabana

2. A continent-wide strategic framework for management of invasive alien plants in Africa
   Gualbert Gbehounou

3. Update status on water hyacinth management in Egypt
   Yahia Hussein Fayad

4. Update status on spread of water hyacinth in the Sudan following the application of biological control
   Ishraga Mohamed Elhassan

5. Update status on water hyacinth in Ethiopia: status and challenges in Upper Blue Nile River
   Wassie Anteneh

6. Update status on water hyacinth infestation, distribution, impact and management strategies in Lake Victoria, Kenya
   Reuben Omondi

7. Update status on water hyacinth management in Tanzania
   Msami Elibariki

8. Biological control of weeds by using plant pathogens
   Yasser Shabana

All the following presentations have been inserted as received from authors. The editors take no responsibility for errors.

Can biological control really work in diverse weed systems?

Yasser Shabana

Weed Systems

- Water hyacinth (floating aquatic weed).
- Hydrilla (submerged aquatic weed).
- Sunflower broomrape (root parasitic weed).
- Common waterhemp (crop weed).

System 1: Water Hyacinth, *Eichhornia crassipes*

Water Hyacinth - The Problem

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قلب مصر 11 10 قلب مصر

ورد النيل.. القاتل الناعم

يؤدى لمزيد من الفقر المائى

يستهلك ٢ مليارات متر مكعب من المياه

ويدمر الثروة السمكية والكائنات البحرية

المكافحة صورية.. والمزارعون يعانون أزمة الرى

القمامة تتحالف مع الورد فى اعتراض جريان النيل!

ياريت الحكومة تاخد الورد وتترك لنا الميه.

ورد النيل يغطى الترع بشمال الشرقية!

رغم اعتماد ٢٠ مليون جنيه للمقاومة سنويا

بسبب الرى والصيد وحركة المعديات بالشلل

الصيد محظور فى مناطق ورد النيل ببحيرة المنزلة

شريان الحياة «يتمزق» فى رشيد

كراكة واحدة للمكافحة بـ٦٠ كيلو مترا.. والعطش يهدد زراعات الصيف

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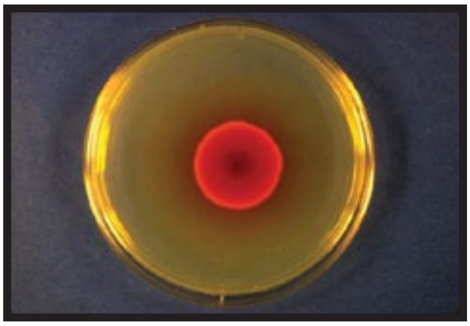
Alternaria eichhorniae 5 (Ae5)



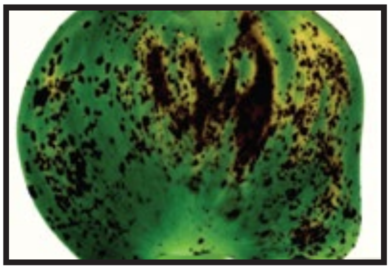
- *Alternaria eichhorniae* produces a bright red pigment in the medium.
- Compare with *A. alternata.*

Shabana,1992

A. eichhorniae is Pan-African

- *A. eichhorniae* was reported on Water hyacinth in 8 African countries.
- Therefore, a mycoherbicide based on *Alternaria eichhorniae* could be used in Africa without major phytosanitary regulation problems.

Disease Symptoms Caused by Ae5



Shabana *et al.,* 1995

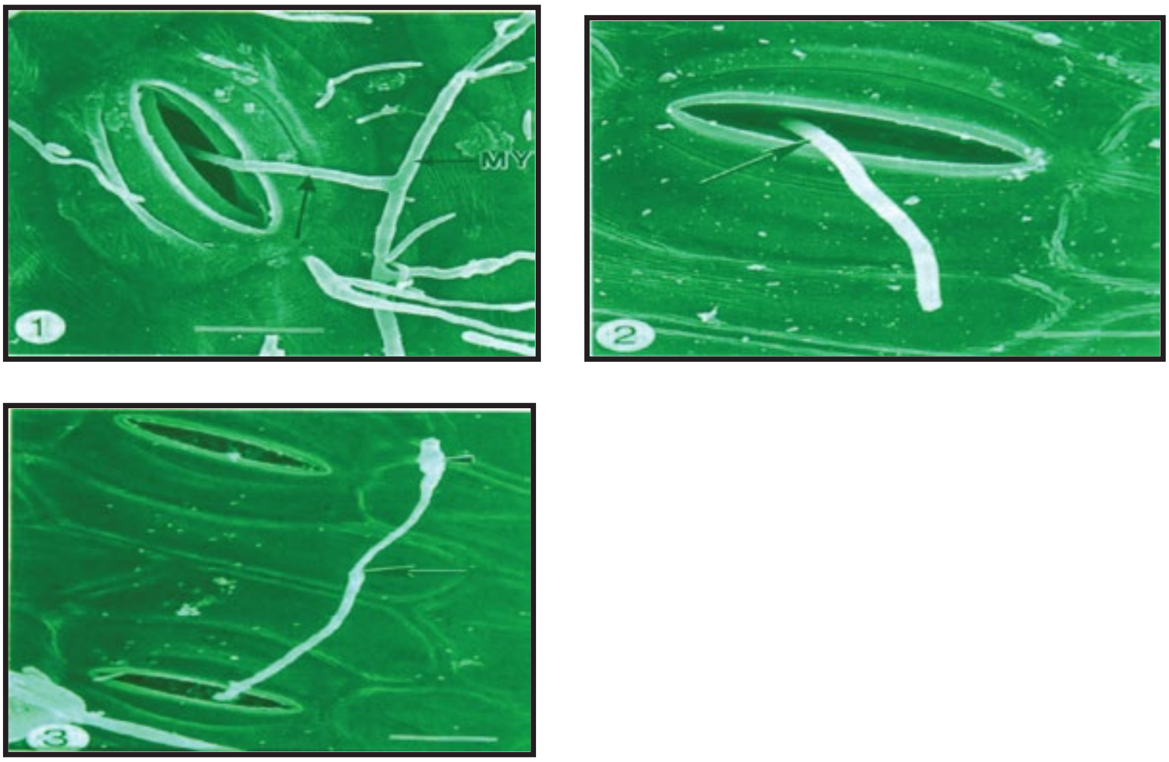
Attempts to Improve the Efficacy of Ae5

- Sporulation.
- Phytotoxin production.
- Optimum conditions for highly-virulent inoculum.
- Epidemiological requirements for high DS.
- Physiological and ultrastructural host responses.
- Mycoherbicidal formulation.

Important Findings

- The efficacy of spore inoculum was equal to mycelial inoculum.
- At least 10h of continuous dew was required for a high level of weed control.
- *A. eichhorniae* proved to be host- specific to water hyacinth.

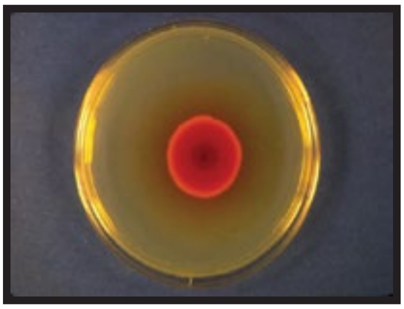
Penetration of Water Hyacinth Leaves by Ae5



Shabana *et al.*, 1997

Mycoherbicidal Formulations

- In hydrophilic polymers.
- In invert emulsions.
- In vegetable oil emulsions.

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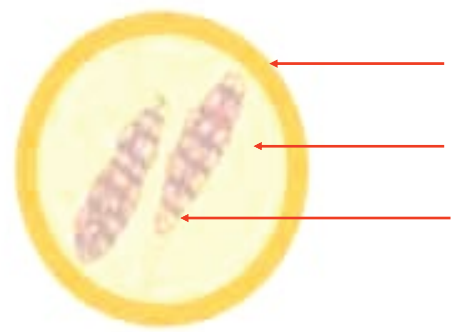
The Invert Emulsion Formulation



Effect of Ae5 Formulated in Cottonseed Oil Emulsion on Water Hyacinth in Field Plots

Treatment	DS (%) after		
	3 wk	5 wk	7 wk
Noninoculated control	3 b [a]	15 b	30 b
Inoculated	70 a	90 a	100 a

[a]Means within a column followed by the same letter are not significantly different a ccording to Duncan's multiple range test (P=0.05).

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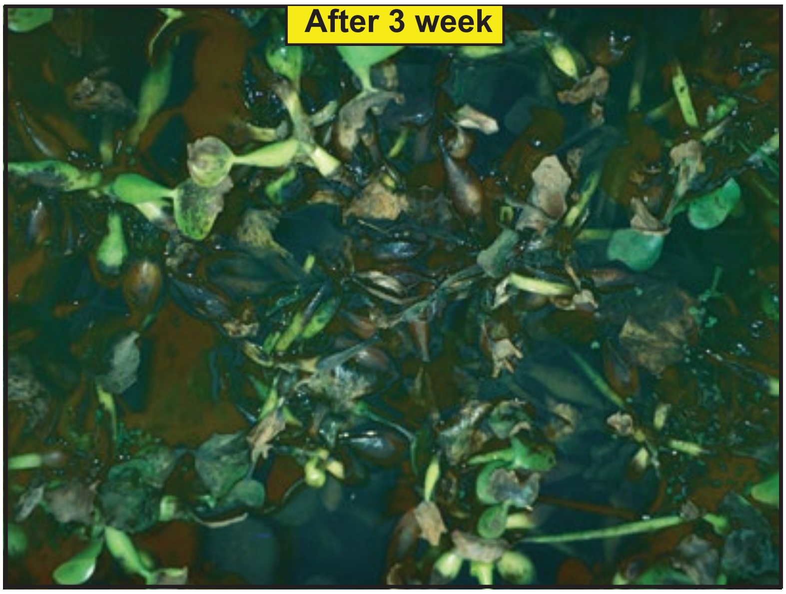

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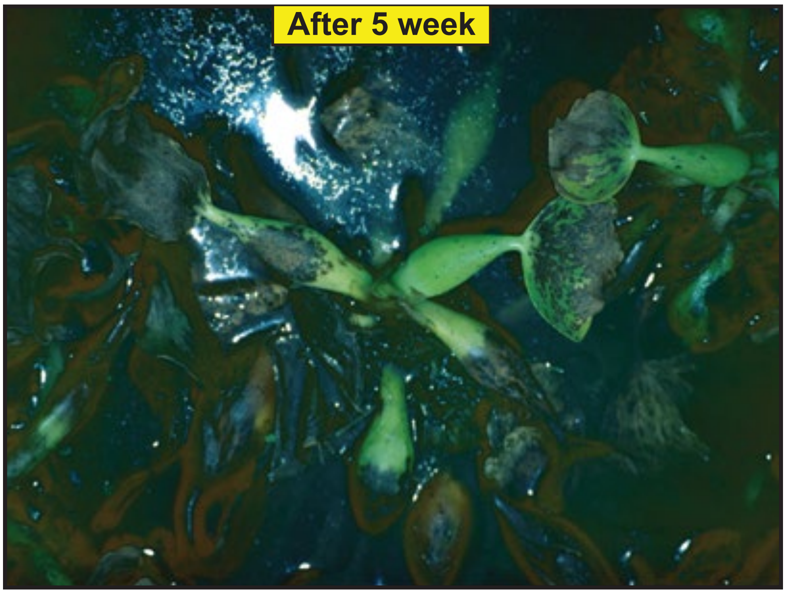

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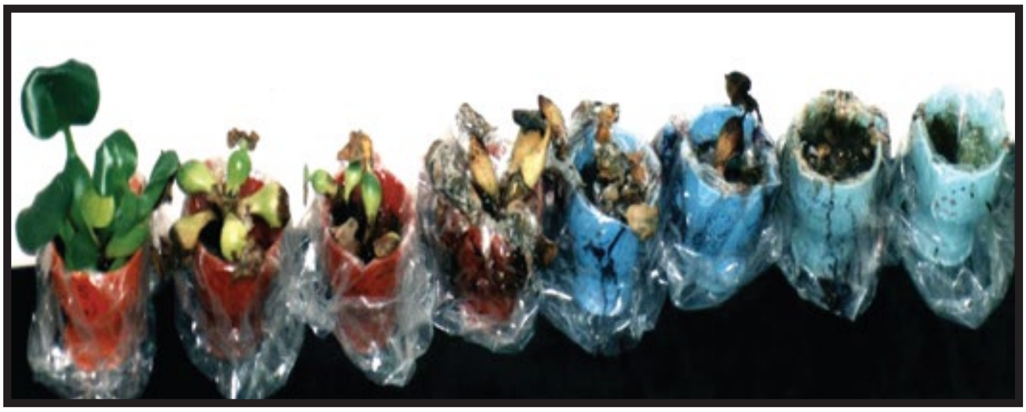
Using Ae5 in Integration with an Enzyme Inhibitor

- To further improve the biocontrol efficacy of Ae5, integration with 3, 4-methylenedioxy trans-cinnamic acid (MDCA), a phenylpropanoid pathway inhibitor that weakens the plant's defense system, was explored.

Shabana 2005. Biocontrol Science & Technology 15: 659-669.



Control	MDCA Only 1 *mM*	MDCA Only 2 *mM*	Ae5 Only	MDCA + Ae5 1 *mM* Time 0	MDCA + Ae5 1 *mM* 12 days before Time 0	MDCA + Ae5 2 *mM* Time 0	MDCA + Ae5 2 *mM* 12 days before Time 0

Shabana 2005. Biocontrol Science & Technology 15: 659-669.

Bio-Safety of Ae5 against Fish

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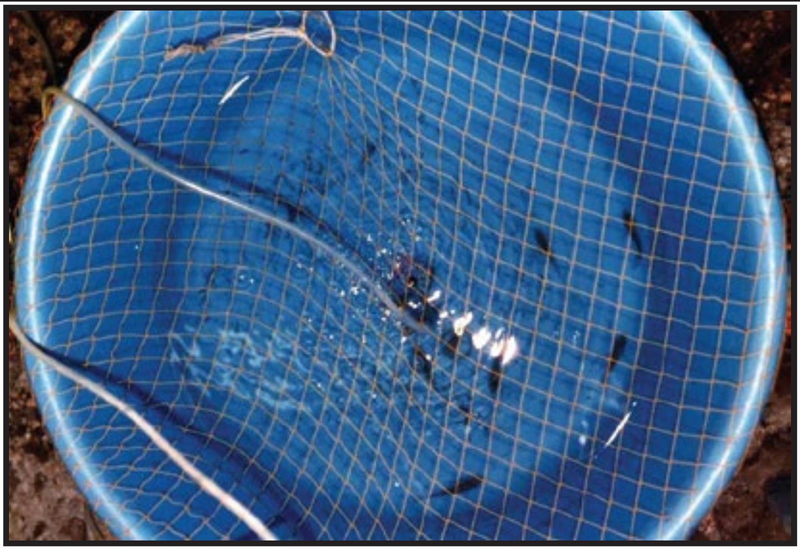

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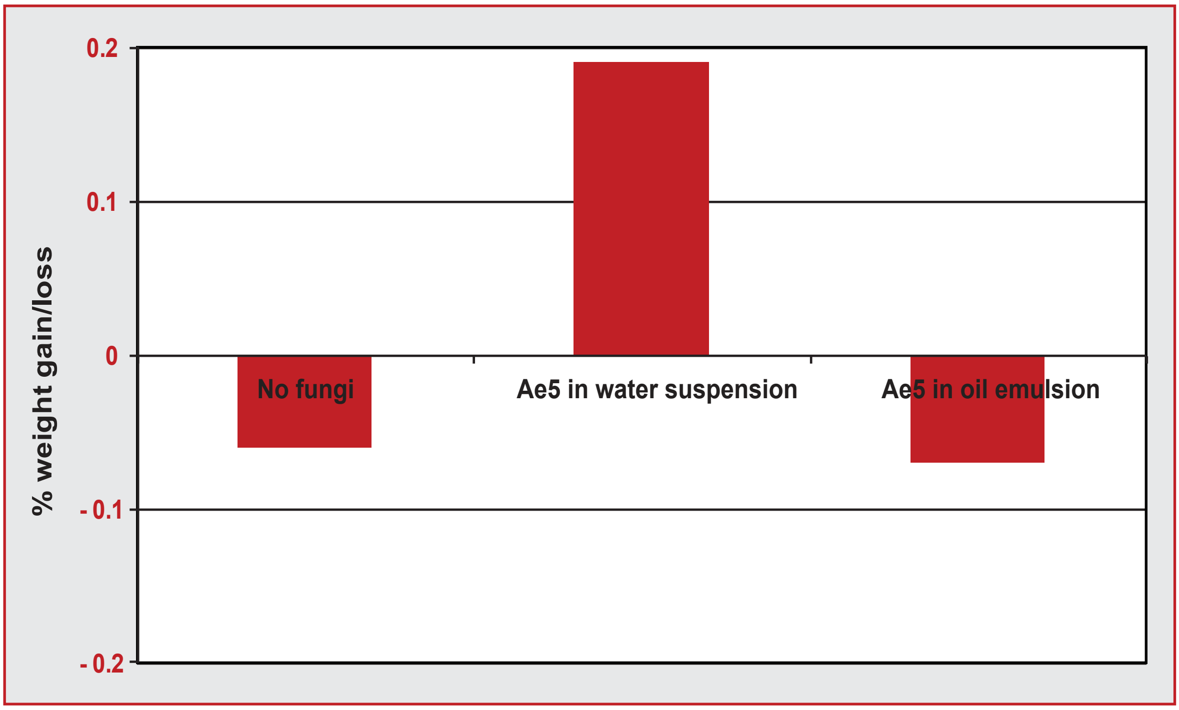
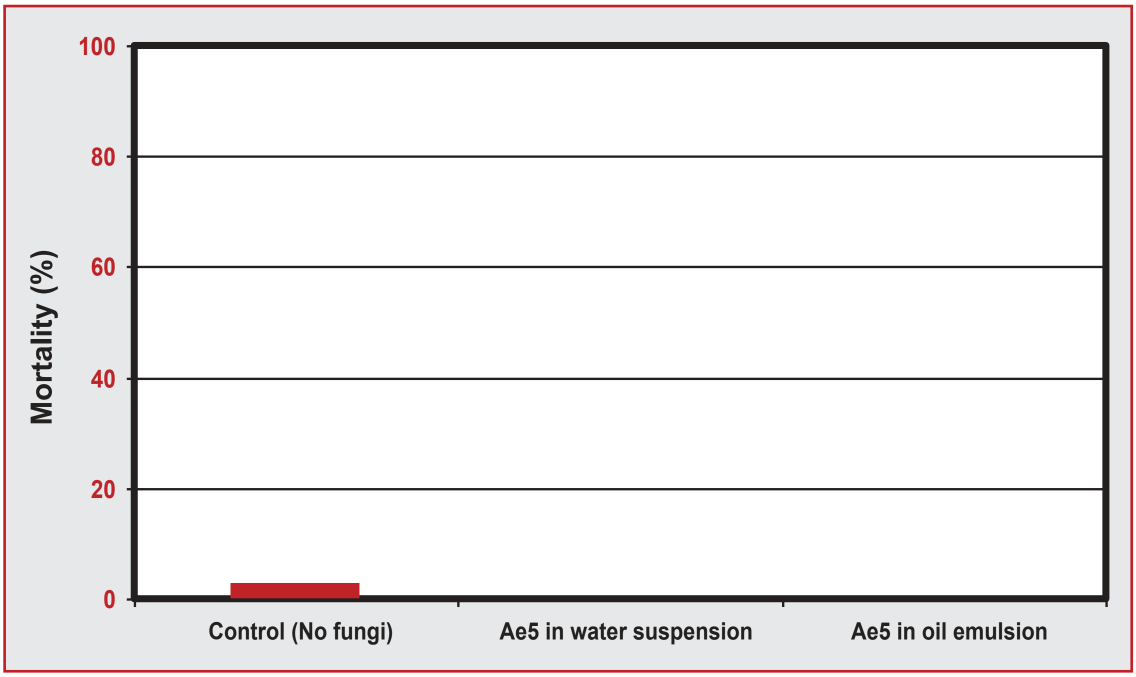
- Small - size fish (~ 3 g).
- 3 treatments: Ae5 in water, Ae5 in oil, and check.
- 4 replicates were used.

Effect of Ae5 on weight gain/loss of small - size Nile Tilapia fish



Effect of Ae5 on fish mortality using small - size Nile tilapia fish



Bio-Safety of Ae5 against Rats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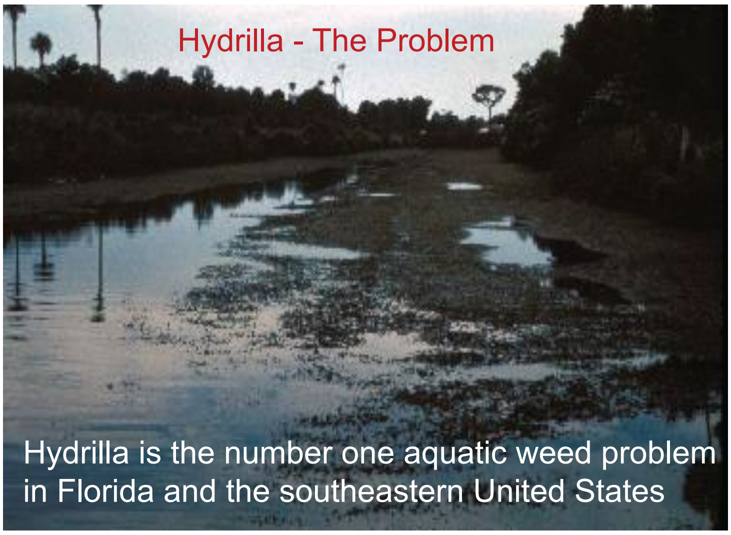
Conclusions

- Ae5 is a virulent and host-specific bioherbicide agent for water hyacinth.
- It can be easily cultured.
- Infection can take place either from mycelia or conidia or a suitable formulation.
- Ae5 is safe and can be used for the biocontrol of water hyacinth with no fears.
- Invert emulsion or vegetable oil emulsion formulations can effectively avert dew dependence.
- The latter is readily available in the marketplace and easier to apply in the field.
- The disease severity by Ae5 increased when applied to water hyacinth plants pretreated with the enzyme inhibitor, MDCA.

System 2: Hydrilla, *Hydrilla verticillata*



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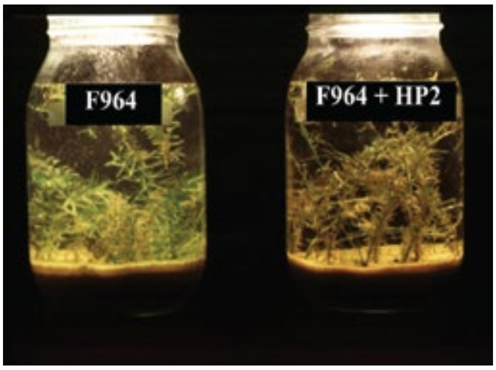
Findings

- During a one year survey, 2200 microbes were recovered from hydrilla,surrounding water, and sediment collected from 10 man-made ponds and three natural lakes in Florida.
- Acremonium sp., Botrytis sp., Curvularia sp., Cylindrocarpon sp., Fusarium moniliforme, and Fusarium culmorum were capable of killing hydrilla in tube assays.The latter was the most effective one.
- For maximum bioherbicidal activity of Fusarium culmorum against hydrilla, it should be applied ata rate of 5×10^5 conidia/ml at 20 ^oC to 25^{o}C water temperature.

Shabana & Charudattan 1996. J.Aquatic Plant Manage. 34: 60-68.
Shabana *et al.* 2003. J.Phytopathology 151: 607-613.

Integrated Control of Hydrilla

- *Fusarium culmorum* (F964) and the leaf-mining fly, *Hydrellia pakistanae* were tested singly and in combination for their capability to kill hydrilla .



Shabana *et al*. 2003. *J.Aquatic Plant Manage*. 41: 76-81.

Conclusions

- The damage level caused by the combination Fusariam culmorum / Hydrilla pakistanae were 3 times greater compared to the insect alone.
- This combination appears a promising approach for integrated control of hydrilla.

System 3: Sunflower Broomrape, *Orobanche cumana*

Orobanche cumana - The Problem

- Severe infestation lowers not only seed yield but also the seed's oil content.
- 100% yield loss may occur by heavy infestations.

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Why Biological Control?

- No hand weeding is practised.
- No chemical herbicides in commercial use .
- No durable resistance in sunflower.
- Biological control may be a promising solution.

Fusarium oxysporum f. sp. orthoceras (FOO), Pathogenicity to O. cumana

Disease Symptoms:

- Wilting.
- Die back.
- Brown blotches.

Shabana *et al.* 2003. Biological Control. 26: 95-108.

Fungal Formulations

Alginate granules

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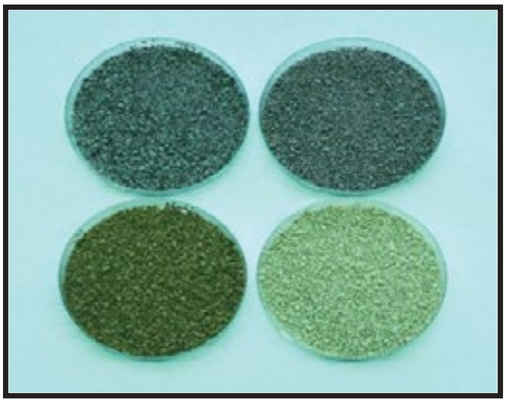
Pesta granules
(Pesta = Pasta + Pest)



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Standard Pesta Ingredients

- Semolina (durum wheat flour).
- Kaolin.
- Fungal propagules (active ingredient).
- DI Water.

How do we produce Pesta granules?

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Pesta Formulations

19 Pesta formulations were prepared using:

- **8 adjuvants:**
- sucrose, glycerol, yeast extract, stillage, sodium alginate, corn flour, waterlock®, cellulose.

- **2 types of fungal spores:**
- microconidia, chlamydospores.

Efficacy of Pesta Formulations

Treatment	% Disease Severity [a]	% Shoots Produced Seeds	% Reduction in Orobanche Biomass
PM 1	97.9 a	0 b	75.7
PM 5	97.8 a	0 b	71.4
PM 7	96.6 a	11.6 b	80.0
PC 14	99.4 a	0 b	80.0
PC 15	97.1 a	12.5 b	66.9
C -	1.3 c	93.5 a	0

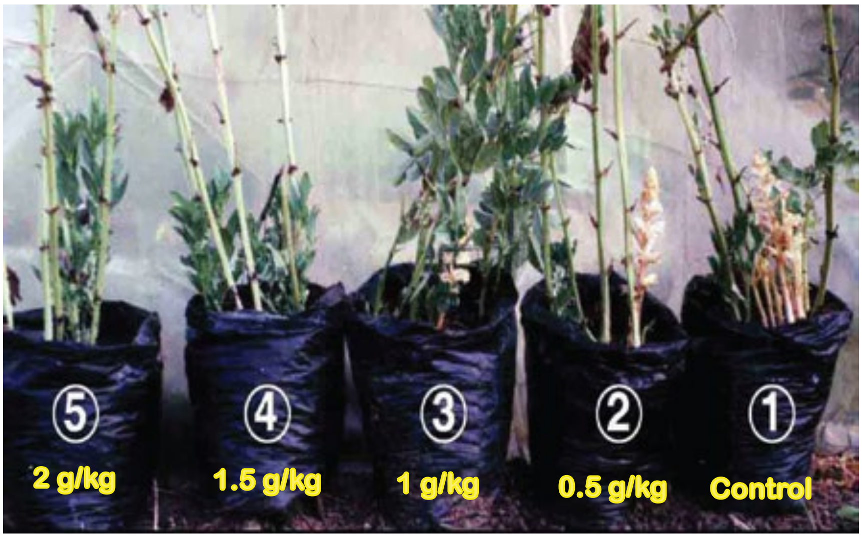
[a] Disease severity = the amount of disease on Orobanche shoot.
[b] Values in each column followed by the same letter(s) are not significantly different according to Duncan's new multiple range test (P = 0.05).

Shabana *et al.* 2003. Biological Control 26: 95-108.

Other Finding

- The mycoherbicidal Pesta increased the sunflower seed yield by 5-11 times in comparison with C-.

Fusarium oxysporum as a biocontrol agent for *O. crenata*



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System 4: Common Waterhemp

Common Waterhemp (*Amaranthus tuberculatus*) - The Problem

- Wides pread herbicide resistance in waterhemp.
- Water hemp emerges later in the season than most other weed species.


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Disease Symptoms Caused by *Microsphaeropsis amaranthi*


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Objectives

- Develop low-cost substitutes for expensive laboratory media.
- Develop methods for the mass-production of high quality inoculum.

Materials & Methods

- 18 solid substrates, including seeds and shoots of various plants were tested.
- **Seeds:** rice, wheat, barley, chickpea, lima beans, red kidney beans, pinto beans, navy beans, and lentils + cornmeal.
- **Shoots:** corn, soybean, redroot pigweed, common waterhemp, Timothy hay + corn husks & pine shavings.
- Multiharvest method using trays of V-8 agar exposed to CL or DL was also tested.

- 25 g seeds + 5 g sand + 25 ml DI water

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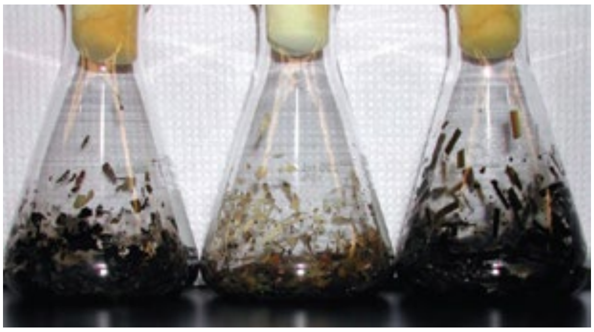
- 1 g leaves + 2 g stems + 3 g sand + 10 ml DI water



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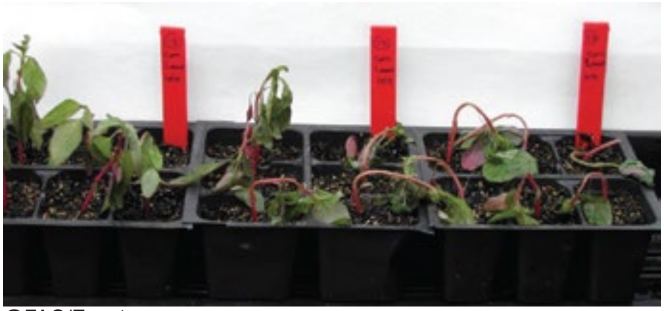
- Conidia were harvested after 4 weeks and 12 weeks.
- Spores' yield on each substrate was determined and virulence was measured by spraying on waterhemp.

Findings

- The best substrate for conidia production was Timothy hay but conidia produced on this medium had low virulence.
- Conidia produced on corn leaves & stalks were significantly more virulent than those produced on any other medium. They caused 100% weed kill within 2 days in the greenhouse.
- Seedlings were more susceptible to Microsphaeropsis amaranthi than mature plants.
- Conidial inoculum was more virulent than mycelial inoculum.

Disease severity caused by *Microsphaeropsis amaranthi* conidia produced on corn leaves & stalks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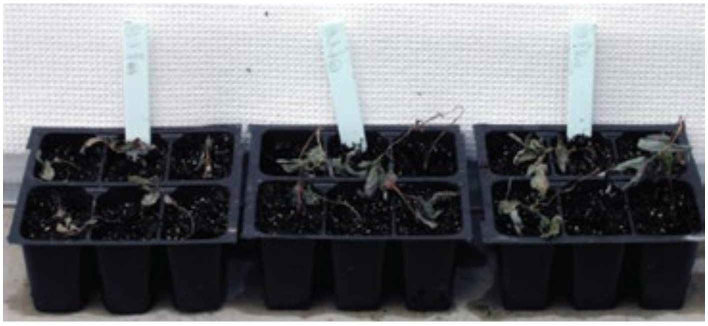
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Acknowledgments

For Water Hyacinth Project:

- DANIDA (Denmark), CABI Bioscience (UK), and TWAS (Italy) provided financial support for this research.

- Elwakil, Zein, Baka, and Abdel-Fattah, Mansoura Univ., Egypt and Charudattan, Univ. Florida had significant contributions to parts of the presented work.

For Hydrilla Project:

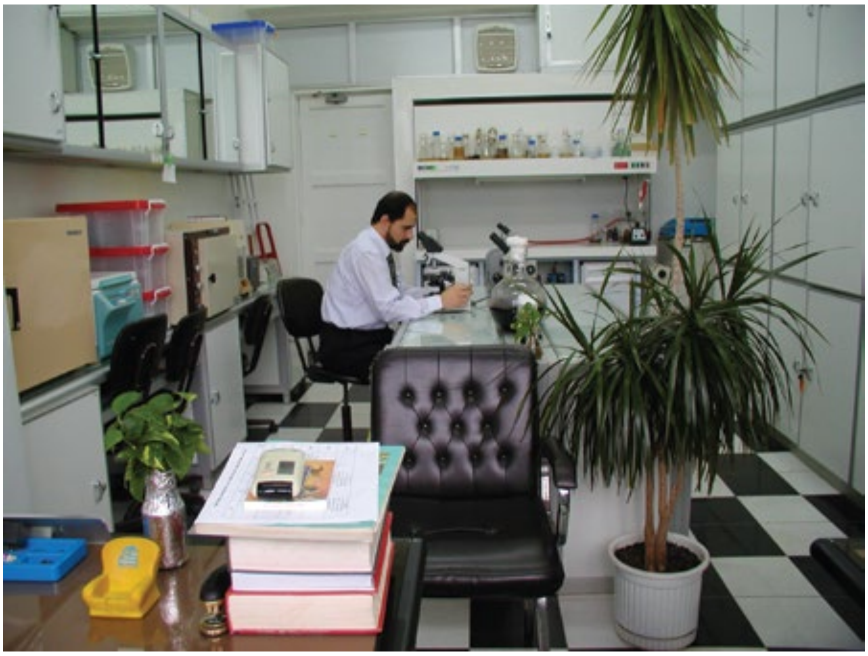
- The project was funded by the USDA-ARS and Univ. of Florida.

For *Orobanche* Project:

- Thanks are due to AvH foundation for offering me a Research Fellowship to accomplish this research at Hohenheim Univ., Germany.

For Waterhemp project:

- Thanks are due to the“Arab Fund Fellowship Program for Distinguished Scholars” for offering me a Research Fellowship to accomplish this research at Purdue University.



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Patent No. 5,393,728

Yasser M. Shabana

INVENTOR

UNITED STATES PATENT

The Commissioner of Patents and Trademarks has received an application for a new and useful invention. The requirements of law have been complied with, and it has been determined that a patent on the invention, known as

BROAD-SPECTRUM BIOHERBICIDE TO CONTROL SEVERAL SPECIES OF PIGWEEDS AND METHODS OF USE

shall be granted under the laws of the United States of America. Therefore, this United States Patent grants to the person or persons having title to this patent the right to exclude others from making, using or selling the invention throughout the United States of America for the term of seventeen years from the date of this patent.

Date of Issue

Attesting Officer

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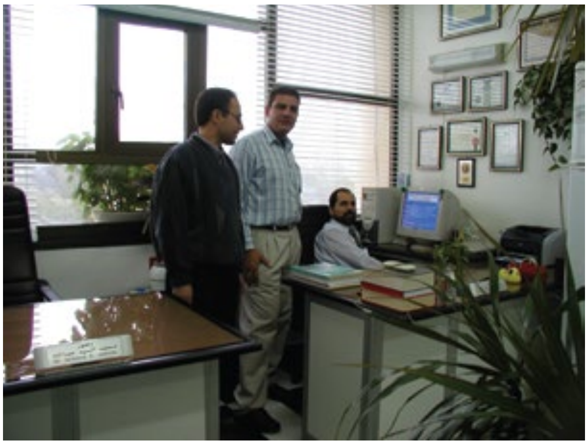

Patent No. 5,510,316

Yasser M. Shabana

INVENTOR

UNITED STATES PATENT

The Commissioner of Patents and Trademarks has received an application for a new and useful invention. The requirements of law have been complied with, and it has been determined that a patent on the invention, known as

PHOMOPSIS SPECIES FUNGUS USEFUL AS A BROAD-SPECTRUM BIOHERBICIDE TO CONTROL SEVERAL SPECIES OF PIGWEEDS

shall be granted under the laws of the United States of America. Therefore, this United States Patent grants to the person or persons having title to this patent the right to exclude others from making, using or selling the invention throughout the United States of America for the term of seventeen years from the date of this patent.

Date of Issue

Attesting Officer

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A Continent-Wide Strategic framework for management of Invasive Alien Plants in Africa

Gualbert Gbèhounou

Overview of presentation
- Invasive Alien Plants.
- Their negative impacts.
- Why a Continental strategic framework?
- Process of development.
- Participants to the process.
- The five pillars.
- Operational structure.
- Next step.

What are Invasive Alien Plants?

- Non native, established, invasive, displacing native species and having negative impacts on the new environment.

Eichhornia crassipes – Water Hyacinth known all over Africa Originally from South America where it has natural enemies thus not a problem there

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Salvinia molesta

Thick mats of the fern *Salvinia molesta* on water bodies are of concern in many African countries

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Parthenium hysterophorus: A new threat in East - Africa

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Solanum elaeagnifolium
A threat to agricultural production in Northern and Southern Africa

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Negative impacts of Invasive Alien Plants

- Displace native species thus reduce biodiversity: picture above are quite illustrative.
- Take land out of agricultural production, toxic to cattle, create public health concerns.
- 12 billion Euros annual damage in Europe for IAS.
- Difficult and expensive to control.

Negative impacts of Invasive Alien Plants (C'd)

- *Negative impacts prompted Aichi Biodiversity Target 9 which stipulates "By 2020, invasive alien species and pathways are identified and prioritized, priority species are controlled or eradicated, and measures are in place to manage pathways to prevent their introduction and establishment".*

Why a Continental Strategic Framework?

- Prevention is much cheaper but requires exchange of information across regions.
- Management is much more efficient if addressed simultaneously by neighbor countries.
- Thus, European Union is uniting efforts against IAS.
- Donors prefer efficient management and large scale impact.

Process followed for development of the Continent-Wide Strategic framework

1. Consultant recruited to produce a draft Strategic framework.
2. Draft internally reviewed at FAO.
3. Validation workshop by national experts organized at FAO-RAF in Accra in October 2013.

Participants to validation workshop include experts from

1. Inter African Phytosanitary Council (IAPSC)
2. Burkina
3. Ghana
4. Mali
5. Ethiopia
6. Kenya
7. CABI-Africa
8. FAO-HQ
9. FAO-RAF

The five pillars of the Strategic Framework

1. Coordination and leadership at Continental, Regional and National levels.
2. Policies, laws, regulatory framework for prevention and management.
3. Research and capacity development for IAP prevention and management.
4. Implementation of solutions for preventing and reducing the impact of IAPs.
5. Communication, awareness and information.

Operational structure proposed

1. An African Council on Invasive Alien Species.
2. Eight Regional Councils on IAS hosted by Regional Economic Community where possible:
   - ECCAS COMESA
   - IGAD
   - SADC
   - Northern Africa
3. National Councils of IAS:
   - Inter African Phyto-sanitary Council (AU-IAPSC) should play a leading role in the implementation of the Strategic Framework.
   - National Plant Protection Organisations (NPPOs) should act as focal points for leading and coordinating cross-sectoral national actions against IAPs.

A Five Year Action Plan of the Continent-Wide Strategic Framework was also developed

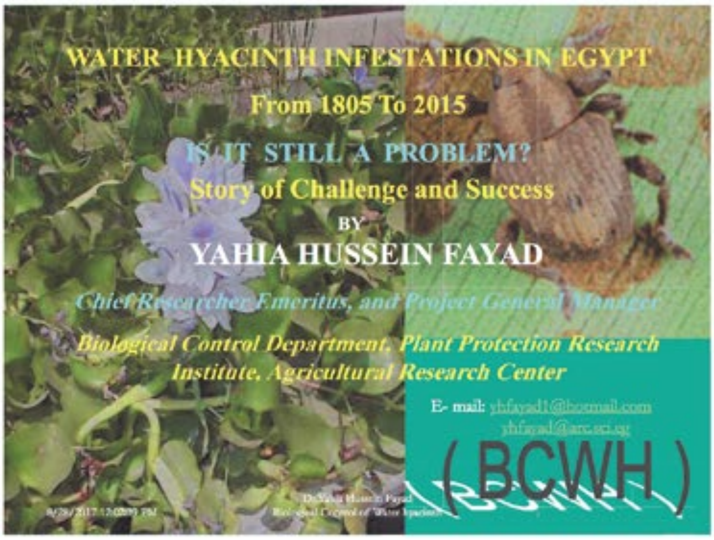
Next steps are:

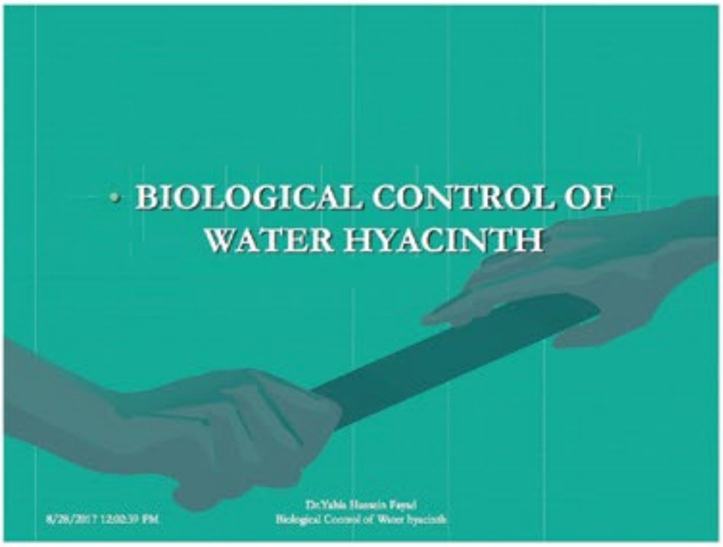
- Formal adoption of the Continent - Wide Strategic Framework by African Union.
- Resource mobilization for implementation of Action Plan.

Please note that current workshop on use of bio- herbicide to control water hyacinth fits under pillar 3 of the Continent-Wide Strategic framework: *Research and capacity development for IAP prevention and management.*

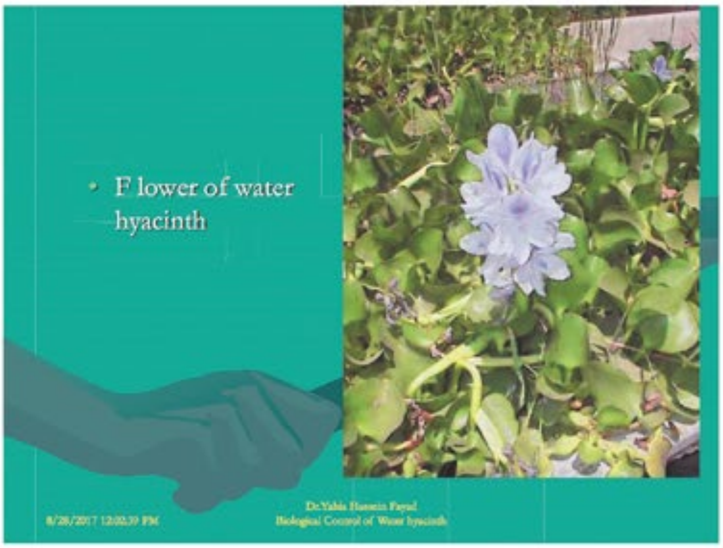
Update status of water hyacinth management in Egypt

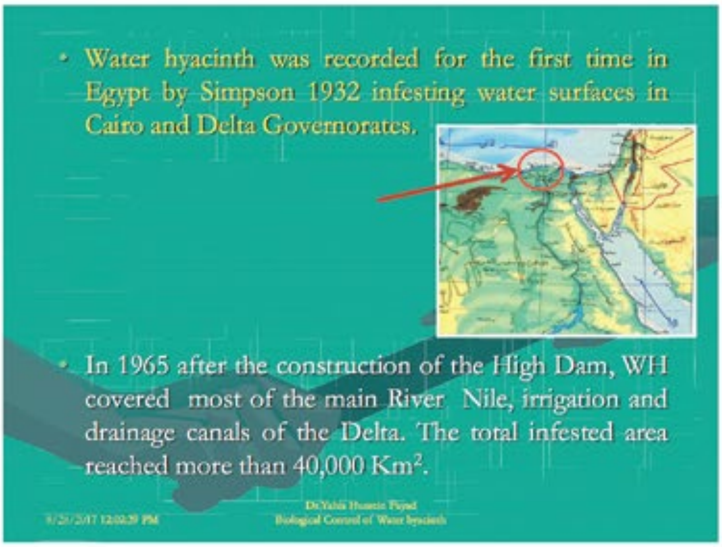
Yahia Hussein Fayad




• BIOLOGICAL CONTROL OF WATER HYACINTH


• Water hyacinth *Eichhornia crassipes* (Mart.), Solms Fam. Pontedriaceae is considered the most serious aquatic floating weed problem infesting fresh water surfaces in the sub tropical and tropical countries.
• It is originated in central and south America.
• The weed was introduced to Egypt During Mohamad Ali Basha Regime (1805-1848) more than 200 years ago as ornamental plant.


• F lower of water hyacinth


• Water hyacinth was recorded for the first time in Egypt by Simpson 1932 infesting water surfaces in Cairo and Delta Governorates.
• In 1965 after the construction of the High Dam, WH covered most of the main River Nile, irrigation and drainage canals of the Delta. The total infested area reached more than 40,000 Km².

Survey Of Water Hyacinth Infestation In Egypt 2000:

Survey conducted all-over the country proved that water hyacinth infestations occur along the main river from Aswan Governorate in the South to Beheira and Demiat Governorates in the North. The weed is common in Cairo, Giza and all Dlta Governorates. Four lagoons; Edko, Mariout, Al Borollos and Al Manzalah (North of Egypt) are heavily infested with WH.

6/28/2017 12:02:39 PM — Dr.Yahia Hussein Fayad, Biological Control of Water hyacinth

ECONOMIC IMPORTANCE OF WATER HYACINTH

***Each plant evapo- transpirates about 0.8 - 1 litter of water daily.**

***The total length of irrigation and drainage net systems reach about 175.000 Km, out of which 6%, (116.000 Feddan), was infested with water hyacinth. This area evapo-transpirates 3 Million Cubic meter daily.**

***Causes serious problems for fisher industry and navigation .**

***Thick mats of water-hyacinth cause depletion of dissolved oxygen reducing fish stocks and disturb wildlife flora .**

***Serves as intermediate host to several species of snails specially Bilharzias and also immature forms of mosquitoes.**

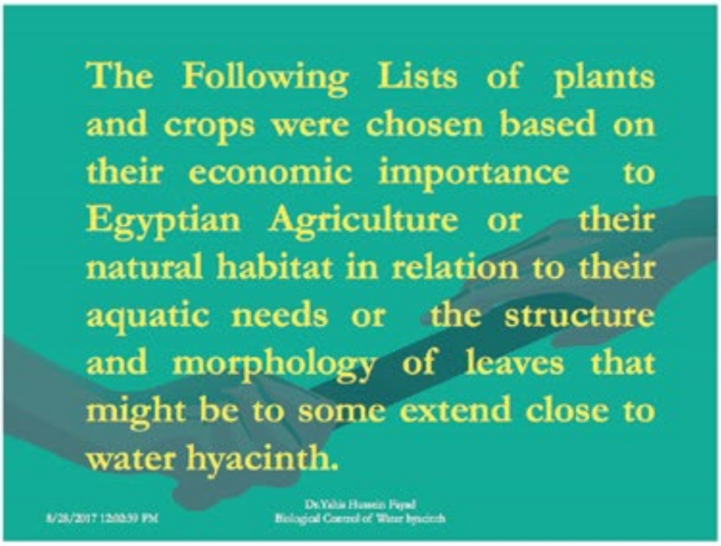
6/28/2017 12:02:39 PM — Dr.Yahia Hussein Fayad, Biological Control of Water hyacinth

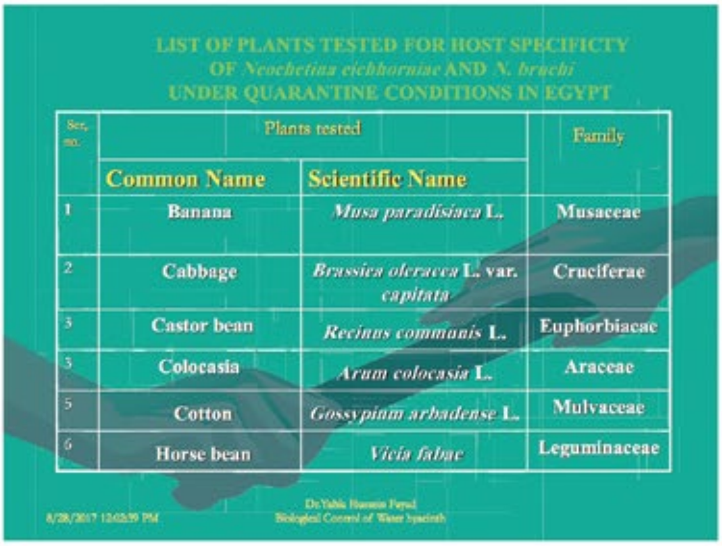
Scientifically, it is well known that when we look for biological control agents of exotic pest, we should go to the origin country of the pest and seek for it's biological control agents.

6/28/2017 12:02:39 PM — Dr.Yahia Hussein Fayad, Biological Control of Water hyacinth

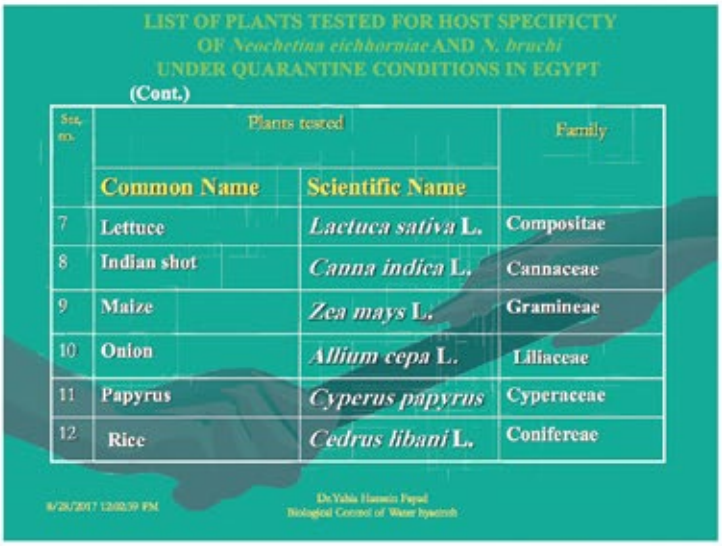
During the period 1978 – 1984 in cooperation with USDA Fort Lauderdale, Florida, 2 curculionids; *Neochetina eichhorniae* Warner and *N. bruchi* Hustache brought from Argentina and reared in Florida were introduced to Egypt and studied under quarantine conditions to determine their host specificity and safety for introduction to other countries for biological control of water hyacinth.

6/28/2017 12:02:39 PM — Dr.Yahia Hussein Fayad, Biological Control of Water hyacinth

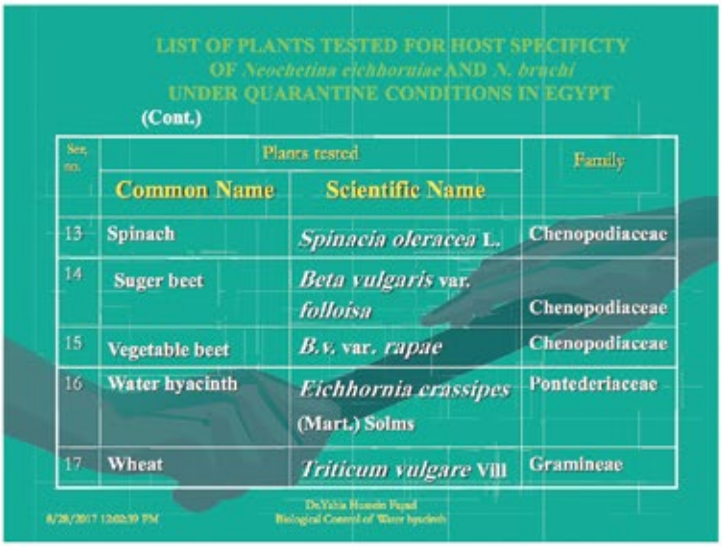


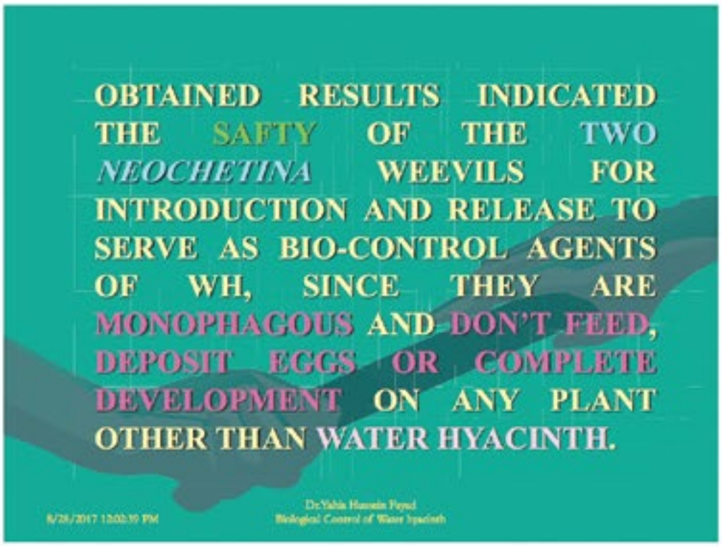

LIST OF PLANTS TESTED FOR HOST SPECIFICTY OF *Neochetina eichhorniae* AND *N. bruchi* UNDER QUARANTINE CONDITIONS IN EGYPT

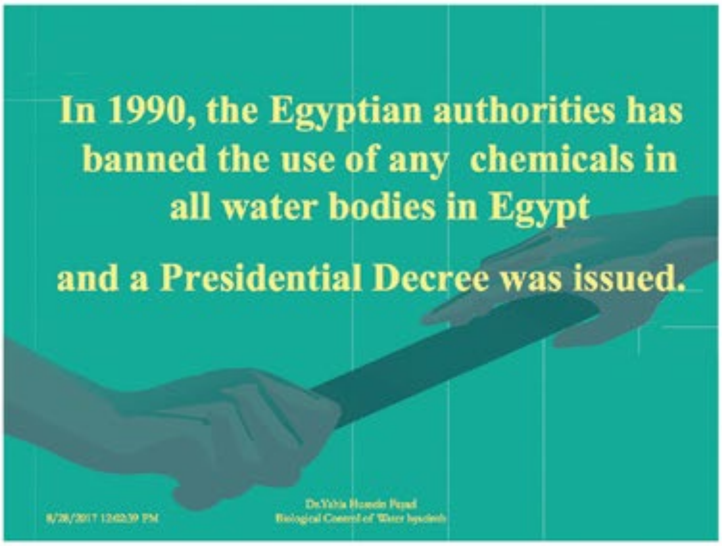
Ser, no.	Plants tested		Family
	Common Name	Scientific Name	
1	Banana	*Musa paradisiaca* L.	Musaceae
2	Cabbage	*Brassica oleracea* L. var. *capitata*	Cruciferae
3	Castor bean	*Recinus communis* L.	Euphorbiacae
3	Colocasia	*Arum colocasia* L.	Araceae
5	Cotton	*Gossypium arbadense* L.	Mulvaceae
6	Horse bean	*Vicia fabae*	Leguminaceae

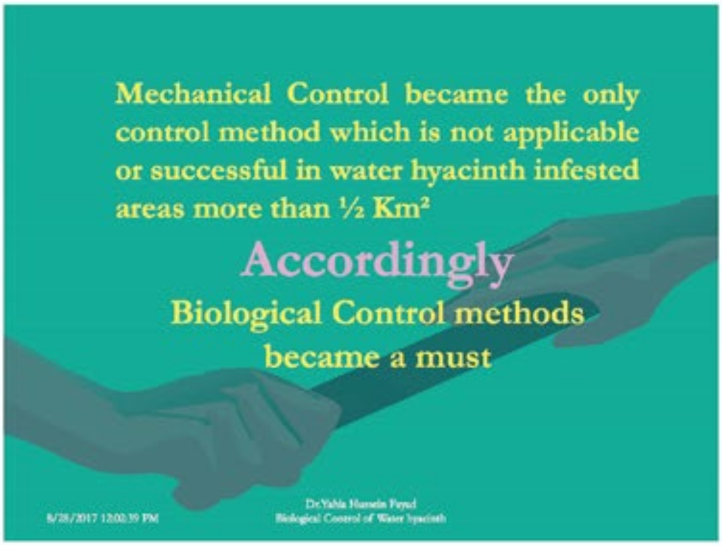

LIST OF PLANTS TESTED FOR HOST SPECIFICTY OF *Neochetina eichhorniae* AND *N. bruchi* UNDER QUARANTINE CONDITIONS IN EGYPT
(Cont.)

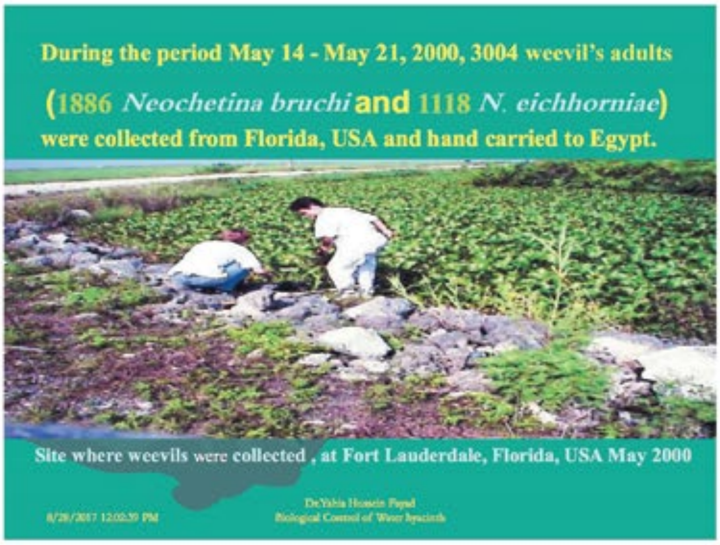
Ser, no.	Plants tested		Family
	Common Name	Scientific Name	
7	Lettuce	*Lactuca sativa* L.	Compositae
8	Indian shot	*Canna indica* L.	Cannaceae
9	Maize	*Zea mays* L.	Gramineae
10	Onion	*Allium cepa* L.	Liliaceae
11	Papyrus	*Cyperus papyrus*	Cyperaceae
12	Rice	*Cedrus libani* L.	Conifereae


LIST OF PLANTS TESTED FOR HOST SPECIFICTY
OF Neochetina eichhorniae AND N. bruchi
UNDER QUARANTINE CONDITIONS IN EGYPT
(Cont.)
Ser. no.
Plants tested
Common Name
Scientific Name
Family
13 Spinach Spinacia oleracea L. Chenopodiaceae
14 Suger beet Beta vulgaris var. folloisa Chenopodiaceae
15 Vegetable beet B.v. var. rapae Chenopodiaceae
16 Water hyacinth Eichhornia crassipes (Mart.) Solms Pontederiaceae
17 Wheat Triticum vulgare Vill Gramineae

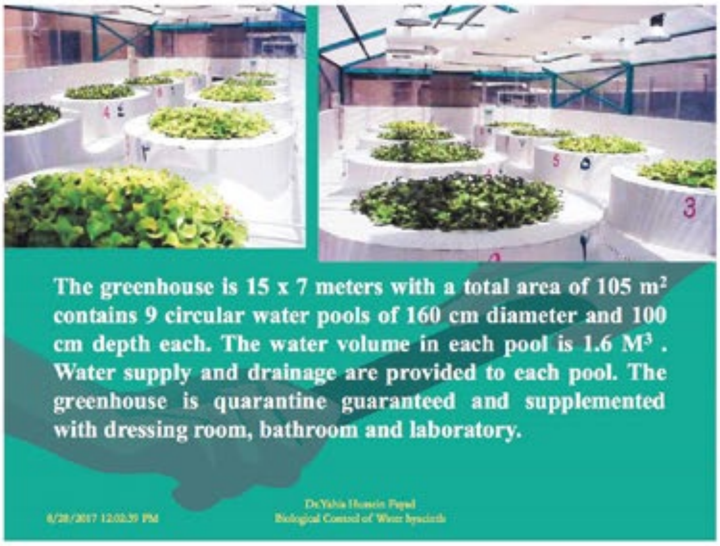

OBTAINED RESULTS INDICATED THE SAFTY OF THE TWO NEOCHETINA WEEVILS FOR INTRODUCTION AND RELEASE TO SERVE AS BIO-CONTROL AGENTS OF WH, SINCE THEY ARE MONOPHAGOUS AND DON'T FEED, DEPOSIT EGGS OR COMPLETE DEVELOPMENT ON ANY PLANT OTHER THAN WATER HYACINTH.

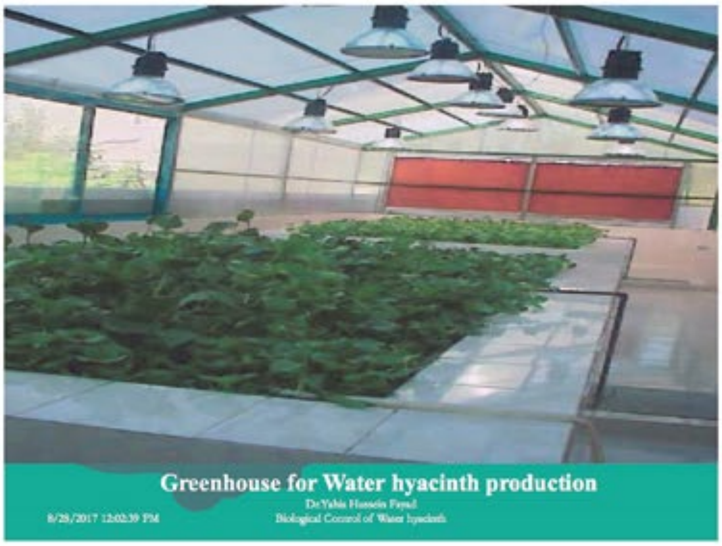

In 1990, the Egyptian authorities has banned the use of any chemicals in all water bodies in Egypt
and a Presidential Decree was issued.


Mechanical Control became the only control method which is not applicable or successful in water hyacinth infested areas more than ½ Km²
Accordingly
Biological Control methods became a must


During the period May 14 - May 21, 2000, 3004 weevil's adults
(1886 *Neochetina bruchi* and 1118 *N. eichhorniae*)
were collected from Florida, USA and hand carried to Egypt.
Site where weevils were collected , at Fort Lauderdale, Florida, USA May 2000
Dr.Yahia Hussein Fayad
Biological Control of Water hyacinth
8/28/2017 12:02:39 PM


Dr.Yahia Hussein Fayad
Biological Control of Water hyacinth
8/28/2017 12:02:39 PM


The greenhouse is 15 x 7 meters with a total area of 105 m²
contains 9 circular water pools of 160 cm diameter and 100 cm depth each. The water volume in each pool is 1.6 M³ . Water supply and drainage are provided to each pool. The greenhouse is quarantine guaranteed and supplemented with dressing room, bathroom and laboratory.
Dr.Yahia Hussein Fayad
Biological Control of Water hyacinth
8/28/2017 12:02:39 PM

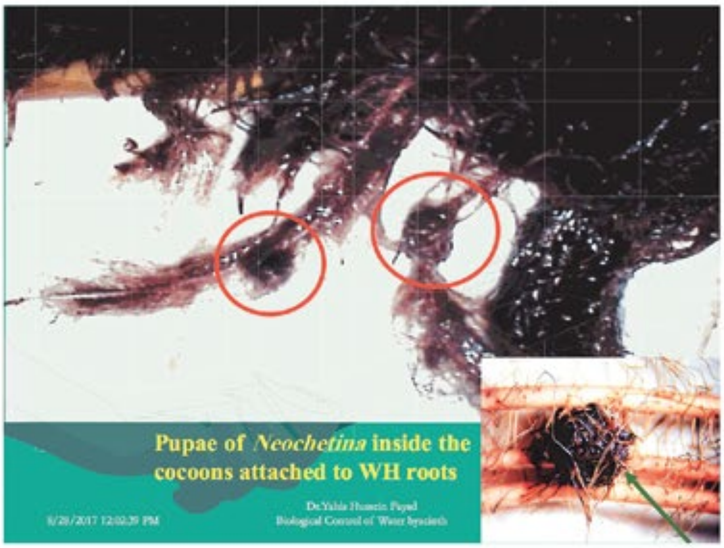

Greenhouse for Water hyacinth production
Dr.Yahia Hussein Fayad
Biological Control of Water hyacinth
8/28/2017 12:02:39 PM


Feeding spots caused by *Neochetina adults*


Deposited Eggs


Larval Damage


Full grown larva just before pupation

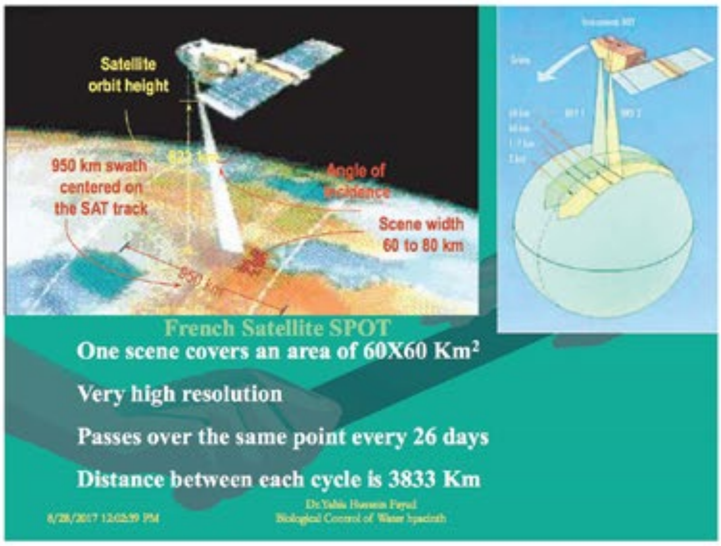

Pupae of *Neochetina* inside the cocoons attached to WH roots

Harvesting of weevils from the pools under the greenhouse (Strainer is used for collecting the weevils)

Total of annually *Neochetina* spp. adults released in the 4 coastal lagoons of Egypt.

Year	Al-Manzalah	Al-Borollos	Edko	Mariout	Total
2000	-	-	4271	2302	6573
2001	-	-	4750	4500	9250
2002	-	-	-	-	-
2003	2000	-	-	3750	5750
2004	3036	2750	-	-	5786
2005	1500	250	-	250	2000
2006	3042	-	-	-	3042
Total	9578	3000	9021	10802	32401

The weevils have been well established after release and widely spread allover the country wherever water hyacinth infestations are exist. Even in sites where the weevils were never released.


Satellite
orbit height
950 km swath
centered on
the SAT track
Angle of
incidence
Scene width
60 to 80 km
French Satellite SPOT
One scene covers an area of 60X60 Km2
Very high resolution
Passes over the same point every 26 days
Distance between each cycle is 3833 Km
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8/28/2017 12:02:39 PM


Mariut Lagoon
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8/28/2017 12:02:39 PM


Water hyacinth infestation in Mariout Lagoon 1999
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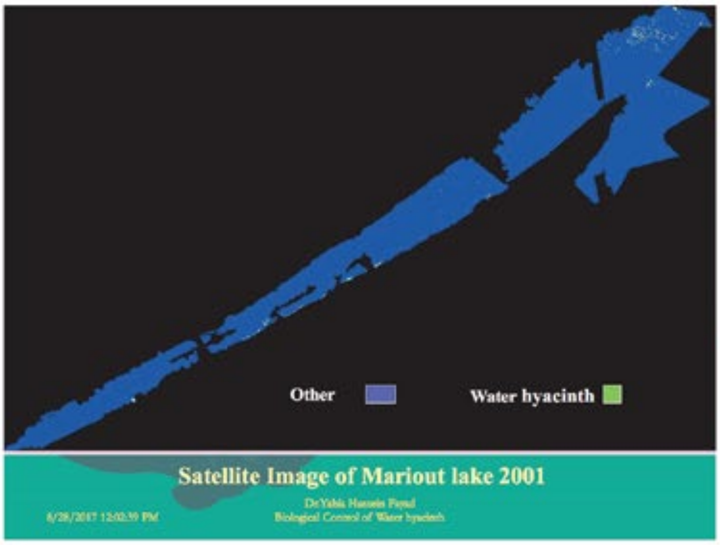
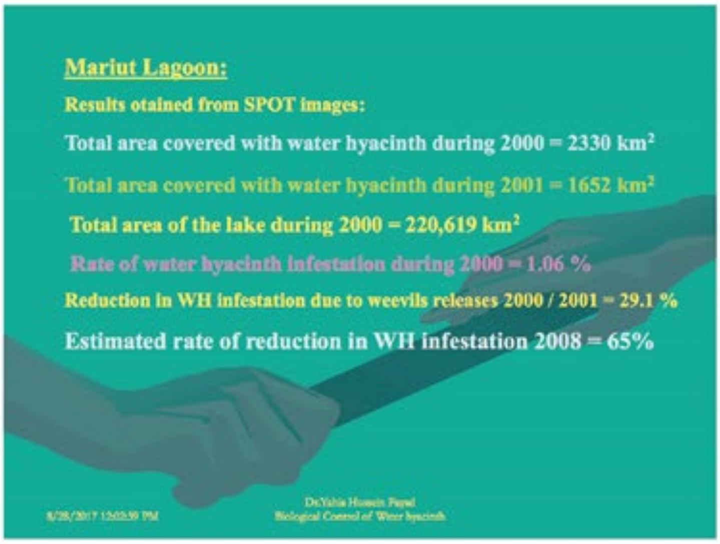
8/28/2017 12:02:39 PM

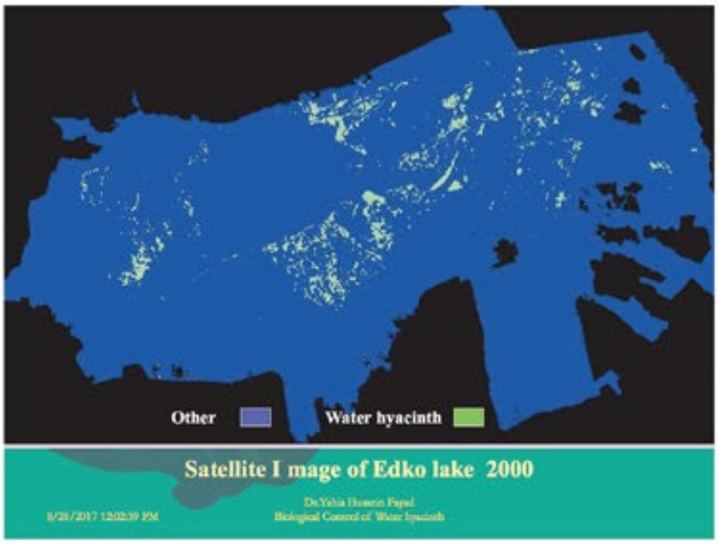

Other
Water hyacinth
Satellite image of Mariout lake 2000 after processing
Dr.Yahia Hussein Fayed
Biological Control of Water hyacinth
8/28/2017 12:02:39 PM

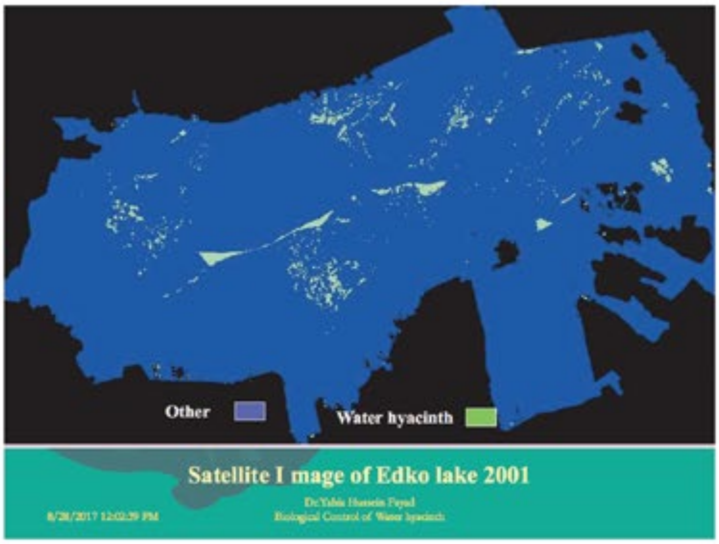

Other
Water hyacinth
Satellite Image of Mariout lake 2001

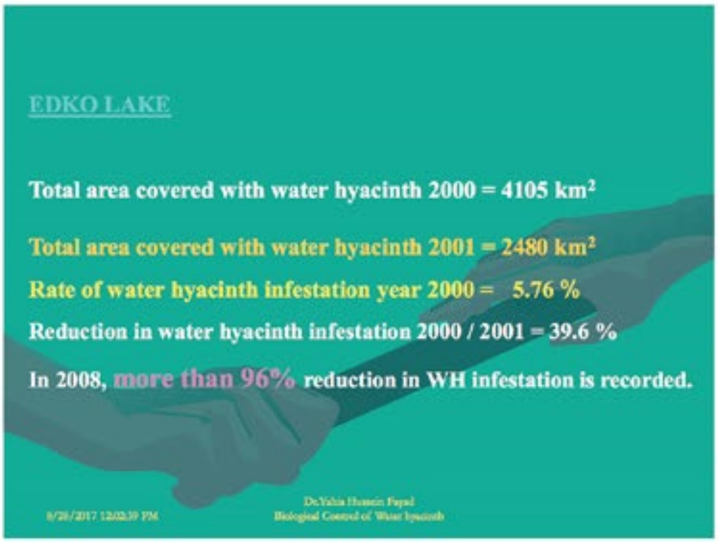

Mariut Lagoon:
Results otained from SPOT images:
Total area covered with water hyacinth during 2000 = 2330 km²
Total area covered with water hyacinth during 2001 = 1652 km²
Total area of the lake during 2000 = 220,619 km²
Rate of water hyacinth infestation during 2000 = 1.06 %
Reduction in WH infestation due to weevils releases 2000 / 2001 = 29.1 %
Estimated rate of reduction in WH infestation 2008 = 65%


EDKO LAGOON

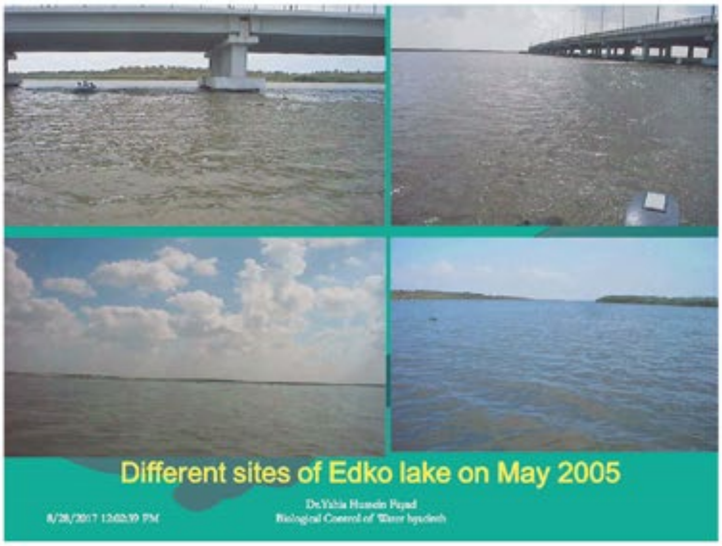

Edko Lake August 1999


Other
Water hyacinth
Satellite I mage of Edko lake 2000


Other
Water hyacinth
Satellite I mage of Edko lake 2001


EDKO LAKE
Total area covered with water hyacinth 2000 = 4105 km²
Total area covered with water hyacinth 2001 = 2480 km²
Rate of water hyacinth infestation year 2000 = 5.76 %
Reduction in water hyacinth infestation 2000 / 2001 = 39.6 %
In 2008, more than 96% reduction in WH infestation is recorded.


Edko lake July 2003


Different sites of Edko lake on May 2005
8/28/2017 12:02:39 PM
Dr.Yahia Hussein Fayad
Biological Control of Water hyacinth


Al Borollos Lake
8/28/2017 12:02:39 PM
Dr.Yahia Hussein Fayad
Biological Control of Water hyacinth

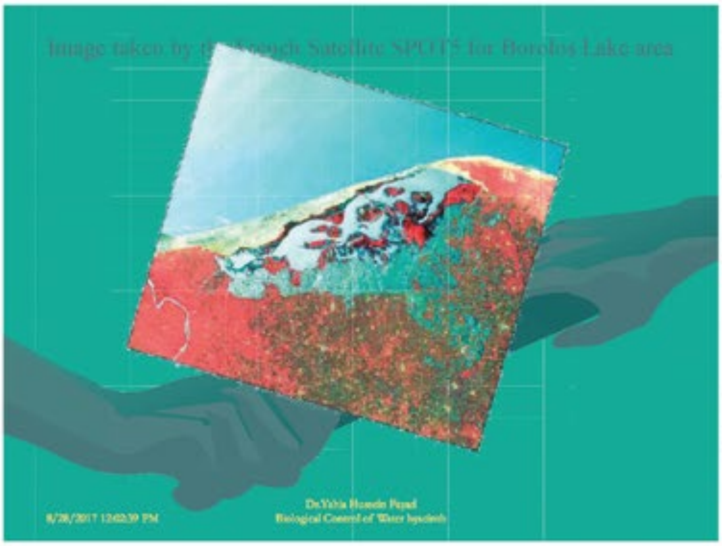
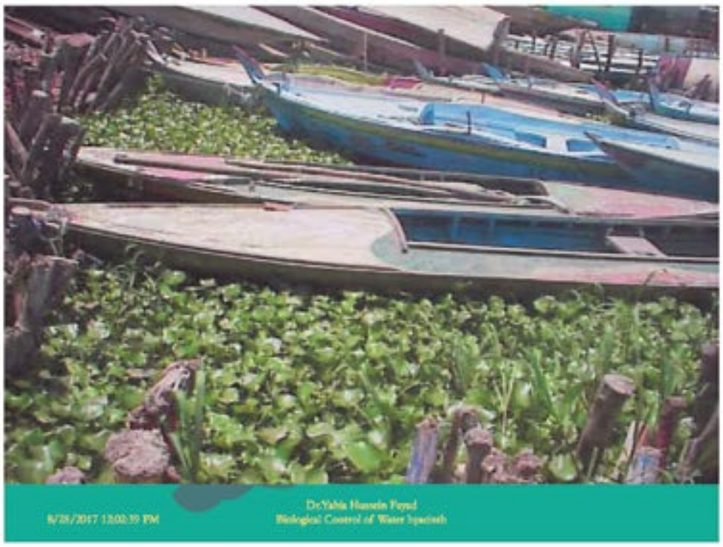
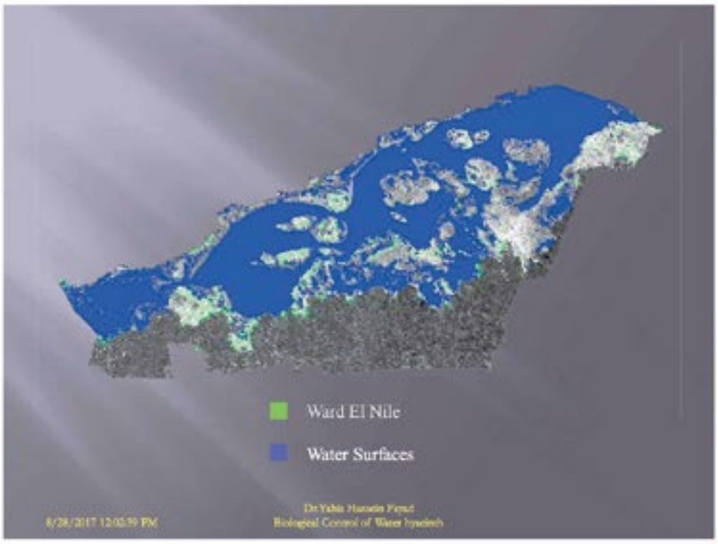


Image taken by
Satellite SPOT5 for
8/28/2017 12:02:39 PM
Dr.Yahia Hussein Fayad
Biological Control of Water hyacinth


8/28/2017 12:02:39 PM
Dr.Yahia Hussein Fayad
Biological Control of Water hyacinth

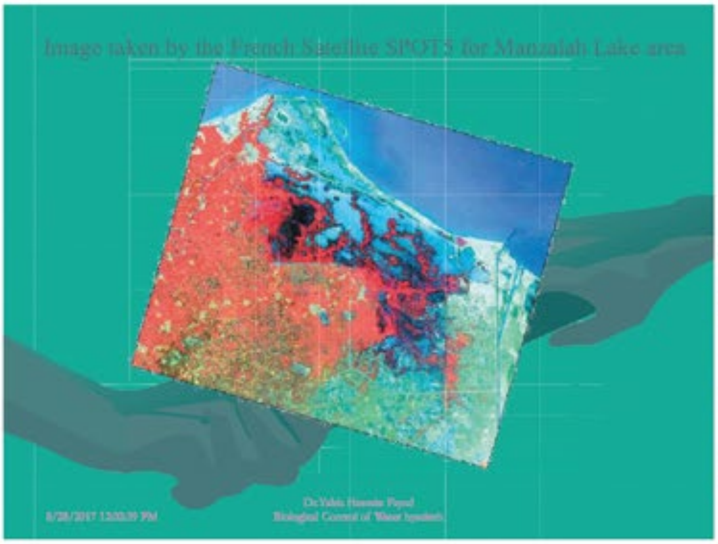


Borollos Lake	Total area of lake (Feddan)	Total area of lake covered by Water Hyacinth (Feddan)	Total area of water surfaces (Feddan)	Ratio between areas of Water Hyacinth & Water surfaces %
April 2000	154004	4983	56731	8.78%
May 2005	156006	2745	58222	4.71%
Percentage of reduction in the areas covered with Water Hyacinth between 2000 & 2005 = 44.91%				

Year	Total area of water hyacinth (Feddan)	Total area of water coverage (Feddan)	Ratio between areas of Water Hyacinth & Water surfaces %
2006	1740.5	64066	2.72%

Percentage of reduction in the areas covered with Water Hyacinth between 2005 & 2006 = 37.04%
To reach a total reduction between 2000 & 2006 = 65.31%.
Estimated Reduction in 2008 ≥ 95%

Al Manzalah Lake



Manzalah Lake	Total area of lake covered by Water Hyacinth (Feddan)	Total area of water surfaces (Feddan)	Ratio between areas of Water Hyacinth & Water surfaces %
April 2000	7861	99590	7.89 %
May 2005	6650	103740	6.41%

Percentage of reduction in Water Hyacinth between 2000 & 2005 = 15.4%

Percentage of reduction in water hyacinth between 2005 & 2006 = 20.98%

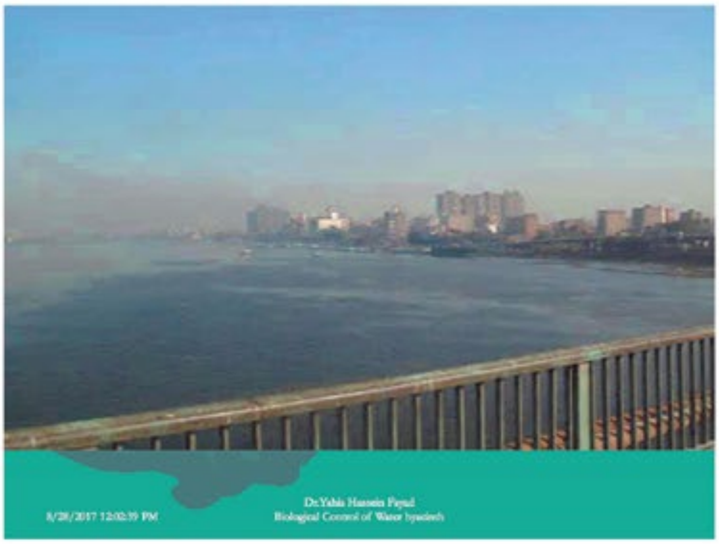
Percentage of reduction between 2000 & 2006 = 33.14%

Estimated Reduction in 2008 = 80%


إصابات ورد النيل بدسوق
محافظة كفر الشيخ
٢٠١٢
Dr.Yahia Hussein Fayad
Biological Control of Water hyacinth


Dr.Yahia Hussein Fayad
Biological Control of Water hyacinth


The River Nile
1 December 2014
8/28/2017 12:02:39 PM
Dr.Yahia Hussein Fayad
Biological Control of Water hyacinth


8/28/2017 12:02:39 PM
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Biological Control of Water hyacinth


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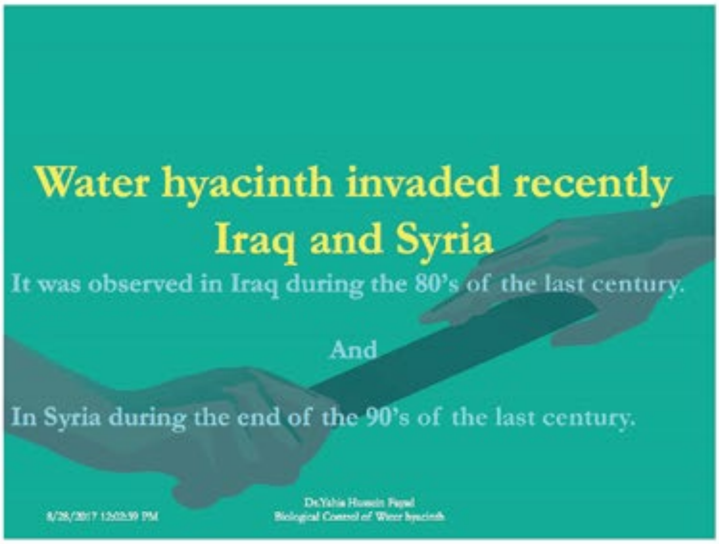
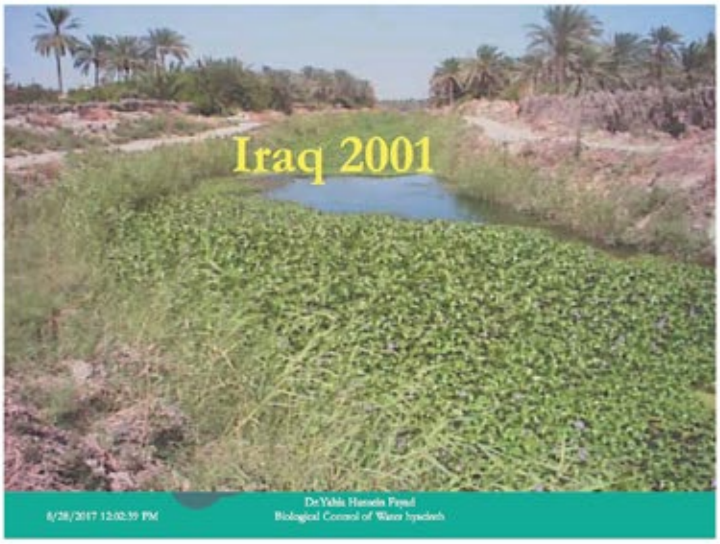
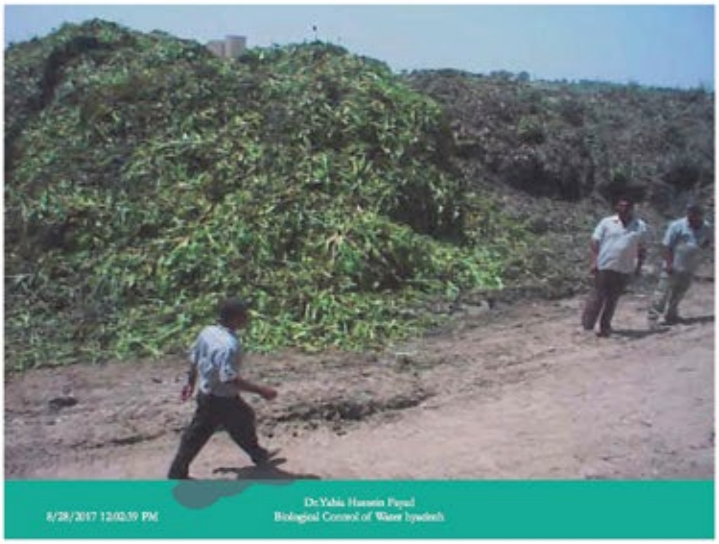
Biological Control of Water hyacinth


Water hyacinth invaded recently
Iraq and Syria
It was observed in Iraq during the 80's of the last century.
And
In Syria during the end of the 90's of the last century.


Iraq 2001


8/28/2017 12:02:39 PM
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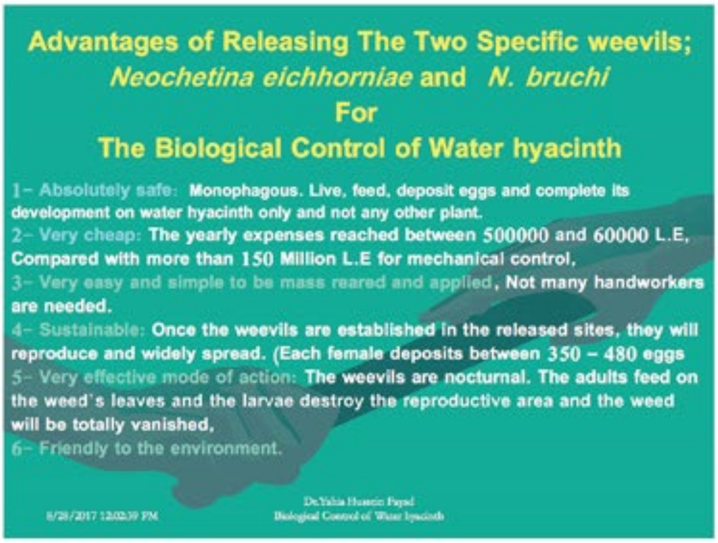
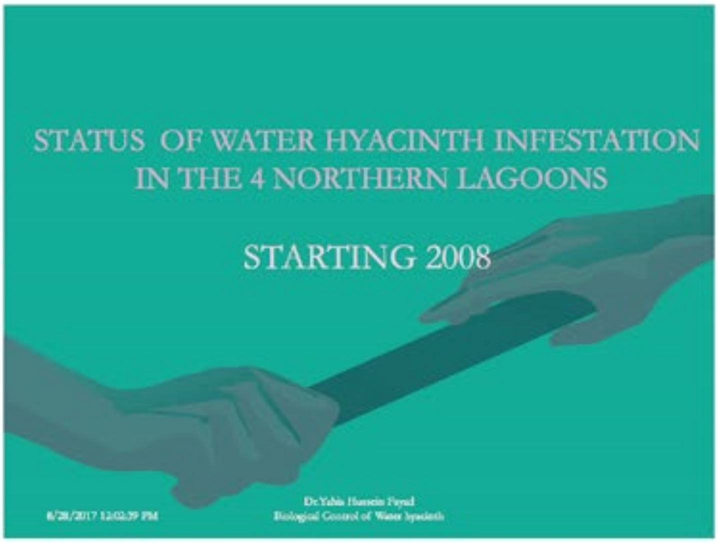
Biological Control of Water hyacinth


إصابات ورد النيل فى سورية ٢٠١٥
8/28/2017 12:02:39 PM
Dr.Yahia Hussein Fayad
Biological Control of Water hyacinth


THE EGYPTIAN INITIATIVE
FOR DEVELOPMENT
IN
WATER BASIN COUNTRIES
Biological Control of water hyacinth
In Sudan 2012
8/28/2017 12:02:39 PM
Dr.Yahia Hussein Fayad
Biological Control of Water hyacinth


6/28/2017 12:02:39 PM
Dr.Yahia Hussein Fayad
Biological Control of Water hyacinth


Advantages of Releasing The Two Specific weevils;
Neochetina eichhorniae and N. bruchi
For
The Biological Control of Water hyacinth
1- Absolutely safe: Monophagous. Live, feed, deposit eggs and complete its development on water hyacinth only and not any other plant.
2- Very cheap: The yearly expenses reached between 500000 and 60000 L.E, Compared with more than 150 Million L.E for mechanical control,
3- Very easy and simple to be mass reared and applied, Not many handworkers are needed.
4- Sustainable: Once the weevils are established in the released sites, they will reproduce and widely spread. (Each female deposits between 350 – 480 eggs
5- Very effective mode of action: The weevils are nocturnal. The adults feed on the weed's leaves and the larvae destroy the reproductive area and the weed will be totally vanished,
6- Friendly to the environment.
6/28/2017 12:02:39 PM
Dr.Yahia Hussein Fayad
Biological Control of Water hyacinth


STATUS OF WATER HYACINTH INFESTATION IN THE 4 NORTHERN LAGOONS
STARTING 2008
6/28/2017 12:02:39 PM
Dr.Yahia Hussein Fayad
Biological Control of Water hyacinth


THE COST OF BIOLOGICAL CONTROL IN THE 4 LAGOONS IS 500.000 L . E . YEARLY
Compared to 150 million L.E every year for mechanical control
8/28/2017 12:02:39 PM
Dr.Yahia Hussein Fayad
Biological Control of Water hyacinth


الأخبار
رئيس مجلس الإدارة
إبراهيـــم سعـــده
رئيس التحرير
جـــلال دويـــدار
أسسها مصطفى أمين وعلى أمين سنة ١٩٥٢
الوزارة أوقفت مقاومة الحشائش بالكيماويات منذ ١٩٨٥
١٥٠ مليون جنيه سنويا لمكافحة ورد النيل
The Ministry of Irrigation spend 150.000.000 L.E. yearly for mechanical control of Water hyacinth
NO COMMENT !!!
8/28/2017 12:02:39 PM
Dr.Yahia Hussein Fayad
10th Arab Congress of Plant Protection, 26-30 October 2009, Beirut, Lebanon


IT IS A STORY OF CHALLENGE AND SUCCESS
8/28/2017 12:02:39 PM
Dr.Yahia Hussein Fayad
Biological Control of Water hyacinth


Thank you very much
8/28/2017 12:02:39 PM
Dr.Yahia Hussein Fayad
Biological Control of Water hyacinth

Update status on spread of water hyacinth in the Sudan following the application of biological control

Ishraga Mohamed Elhassan

Introduction

- Sudan is a very big country, even after separation of South Sudan the area is still big (1 882 000 km^2).
- Sudan is surrounded by eight countries: Egypt, Libya, Chad, Central Africa, South Sudan, Ethiopia, Eritrea and Saudi Arabia, accordingly, too many entry points are scattered on this very long border.
- Population: 33.4 million Peoples, Growing at the rate of 2.46%, Rapid Urbanization, Youth Population.
- Federal System: 17 States.

Farming Systems

A: Irrigated Farming

- Area: 1.9 million ha.
- Main Crops: Cotton, Sorghum, Groundnuts, Wheat, Fruits & Vegetables, Rice, Maze & Fodder.

B: Rain fed Mechanized land Farming

- Area: 7.9 million ha.
- Main Crops: Sorghum, Sesame, Sunflower, Cotton.

C: Rainfed Traditional Farming

- Area: 7.6 million ha.
- Main Crops: Sorghum, Millet, Sesame, Gum Arabic and Groundnut.

Economic Performance

- Average growth rate of 6% of GDP (2011 est.).
- Agriculture provides 44% of GDP, with average growth rate of 4.5% (2011 est.).
- Agricultural exports account for 80% of the total export excluding crude oil.
- Food security indicators has improved.
- Strategic Reserved Food was built.

The White Nile

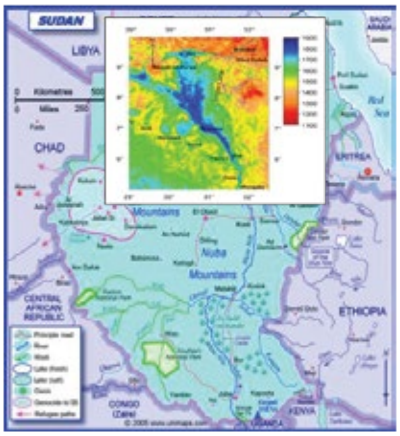
- The Albert Nile flows north to Nimule, where it enters South Sudan and becomes known as the Mountain Nile or Bahr al-Jabal (also Bahr el-Jebel, بحر الجبل), literally "Mountain River".
- The Bahr al-Jabal then winds through rapids before entering the Sudan plain and the vast swamp of the Sudd. It makes its way to Lake No, where it merges with the Bahr el Ghazal and there forms the White Nile.
- An anabranch river called Bahr el Zeraf flows out of the Bahr al-Jabal and flows through the Sudd, to eventually join the White Nile.
- The river flows north into Sudan and lends its name to the Sudanese state of White Nile, before merging with the larger Blue Nile at Khartoum, the capital of Sudan, and forming the River Nile.

Swamp of the Sudd

Water Hyacinth (*Eichhornia crassipes*)

أعشاب النيل (ورد النيل)

- The water hyacinth *E. crassipes* is an aggressive, floating aquatic invasive plant. Classified by IUCN as one of the 100 most aggressive IAS and one of the top 10 worst weeds.
- Native to the northern neotropics of South America, invaded tropic and subtropics as an ornamental plant and still spreading (Morocco).
- In Sudan, was reported near Bor town in the Sudd wetland in 1958 in 2 years it invaded 3700 km^2 of the White Nile and tributaries.



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Water Hyacinth (*Eichhornia crassipes*)

أعشاب النيل (ورد النيل)

- It was breeding all year round in the Sudd wetland between Juba and Malakal then discharged to other river sections.
- Affected biodiversity as the White Nile is sustaining a gene pool of an assemblage of plants, fish, insects and mammals, reptiles etc. Spawning grounds for all the important fish spp.
- Disrupted the socio-economic, cultural patterns and way of life of the Sudd inhabitants are dictated and governed by patterns and dynamics of productivity of wetland.

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Water Hyacinth (*Eichhornia crassipes*)

أعشاب النيل (ورد النيل)

- The Sudd climate is similar to the climate of the amazon basin.
- Water hyacinth has a rapid propagation and morphological characteristics that makes the weed well adapted to rapid distance dispersal and successful colonization of varying habitats.
- Reproduces vegetatively, tolerates a wide range of growth conditions, infested countries across a large range of latitudes and climate.

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Water Hyacinth (*Eichhornia crassipes*)

أعشاب النيل (ورد النيل)

- Exhibited striking phenotypic plasticity.
It spreads rapidly throughout the Sudd region from juba, over a distance of 1700 km to the Jabal Aulia 40 km South of Khartoum.
- Jabal Aulia Dam serves as barrier and with continuous vigilance of the Sudanese authorities Water hyacinth has not established after the dam.
- Control program by Sudanese Egyptian joint Nile committee, was based on herbicidal application by aircrafts and boats.

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The impact of Water Hyacinth on the White Nile

- Interference with river transportation including high operational and maintenance costs for ships.
- Water hyacinth caused many problems by blocking the suction lines of irrigation pumps and clogging canals and other smaller irrigation water channels to irrigate more than 176 schemes with a cultivated area exceeding 110 thousand hectares.
- Water loss by evapotranspiration.
- Displaced all submergent and floating plants.
- Decreased the genetic diversity of the ecosystem (fish spp. extinction).
- High cost of chemical control programs.
- The Cost of chemical control was estimated over US$1 million per annum (Hamdon &Tigani, 1977).

Chemical Control

- Water hyacinth is a highly invasive species and has a tendency to cover and choke major waterways and lake surfaces, which can have numerous detrimental ecological, biological diversity, fisheries, hydroelectric, transportation, and economic results.
- In 1963, The Plant Protection Directorate, deployed 42 boats and three aircrafts and set three stations on the White Nile, to maintain open water access for commercial traffic, and avoid problems of damage to crops by spraying drift.

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Control of water hyacinth was first carried out by applying the herbicide (2,4-D)

Biological control

Background:

1967, The ODM (The Overseas Development Ministry) provided financial support to(CIBC) (Commonwealth Institute of Biological Control) to search for natural enemies of WH in the neotropic sand to rid the environmental from the externalities of chemical control by the herbicide (2,4-D).

- During survey in Trinidad, Guiana, Suriname and the Amazon region in Brazil, potential control agents were encountered. These are:

Natural Enemies of Water Hyacinth

1- *Neochetina eichhorniae* (warrer).
2- *Neochetina bruchi* (hustache) (coleoptera).
3- *Sameodes albiguttalis* (warren) (Lepidoptera).
4- *Acigona infusella* (walker) (Lepidoptera).
5- *Corneps aquaticum* (Bruner) (Orthoptera) grasshopper.
6- *Orthogalumna terebrantis* (walker) (Acri), The Water Hyacinth Mite.

Neochetina bruchi

Neochetina eichhorniae

Acigona infusella

Sameodes albiguttalis

Corneps aquaticum

Orthogalumna terebrantis

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- In 1975 a workshop on aquatic weeds in the Sudan with special reference to Water hyacinth was convened by Sudan Agriculture Research Council of the Sudanese National Council for Research (NCR). University of Khartoum, in collaboration with GTZ, University of Hobenheim, The BODA (British Overseas Development Administration & the USDA (United States Department of Agriculture).


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- 1977 importation of the natural enemies; two weevils, a moth and a mite) started at the faculty of Agriculture, Department of Crop Protection.
- This in collaboration with USDA, CIBC (Common wealth Institute of Biological Control, Headquarter, Trinidad).
- 1978-1979 Screening, mass rearing and then release was made.


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Initiation of program in the Sudan

- The project proposal was prepared in 1976 and funded by ODM/Sudan Government.
- Project initiated on 1979.
- *Neochetina eichhorniae* was imported in Dec.1976 after further host specificity trial at the University of Khartoum, released during May - July 1978.
- CIBC obtained *Neochetina bruchi and Sameodes albiguttalis* in 1980.
- Initial release of *Neochetina eichhorniae,* first instar larvae and eggs were inoculated into the petioles of Water Hyacinth.
- 1979 - 80 release of adults' larvae and infested plants were released in lagoon and side channels where stable mats persisted for several months.

Establishment

- *Neochetina eichhorniae* was first recovered from one of release site near Malakal in June 1979 and was reported widespread by 1981.
- It was collected at Jabal Aulia 270 km down stream from the nearest (1978) release point at Kosti.
- Establishment of *Neochetina eichhorniae* was confirmed April - May 1981. By which time *Neochetina eichhorniae* was present thought the White Nile from Bor to Jabal Aulia, distance 1700 km.
- *Sameodes albiguttalis* release in small numbers at 3 sites, was first recovered in 1982-1983 was widely established from Sobat junction to Kosti and Bor.
- The German Sudanese Water Hyacinth utilization project has had to travel from Taweela to Kosti at regular intervals to collect plants to stock their biogas generators.
- 1982 the weed (Water Hyacinth) was contained, by 1984 Water Hyacinth was removed from the National Pest List; no herbicides were used from that date till now.
- During the course of this endeavor the following was achieved:
- 1/ Training of 4 M.Sc and 2 Ph.D candidates in the field of Water Hyacinth management.
- 2/ Experience.

- The two weevils worked synergistically and did not compete for food.
- The adults produce characteristic feeding scars on the leaves and petioles.
- In the larval stage, the insect tunnels into the petioles and the crown of the plant.
- This feeding results in biotic stress, reduced flowers and seeds, and less vigorous growth.

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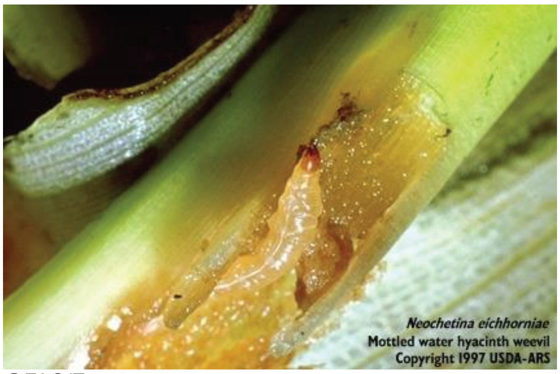
In 1979-80 biological control of Water Hyacinth was applied by introducing these two weevil insects:
Neochetina eichhorniae
Neochetina bruchi

Larva damage of *Neochetina eichhorniae* on water hyacinth's petioles	Adult damage of *Neochetina bruchi* on water hyacinth's leaves



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Larvae and adult of biocontrol insect	Injuries caused by feeding of *Neochetina* species on water hyacinth's leaves

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The Situation Now

- As from 1982 the infestation, receded southwards, now more than 350km of the white Nile are clear.
- The photos are of Jebel Awlia Dam before and after 1982.
- The Nile also retained its biodiversity and the indigenous plants cover more than 95% of the area covered by water hyacinth.

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Biological control of water hyacinth (WH) project, Egyptian initiative for the development of the Nile basin countries

- Project executed and supervised by the Plant Protection Directorate (PPD), of the Federal Ministry of Agriculture, Sudan, in collaboration with the Ministry of Agricultural and Land Reclamation, Arab Republic of Egypt, Agricultural Research Center, Plant Protection Research Institute, Department of Biological Control.
- The proposal aims to reactivate and evaluate a Biological Control (BC) approach which is already existing in Sudan.
- BC is viable, self perpetuating, permanent, cost effective and environment friendly to manage water Hyacinth in the Nile basin.

The ultimate goals of the project are

- To bring the population density of WH to a level below the economics threshold level.
- To conserve the vast amount of water loss (about 7.1 milliard cubic meters annually).
- Advance technical help (natural enemies, capacity building and basic information) in the area of biological control of the weed to other countries of the Nile Basin.
- Enhance capacity of national institution in the biological control technology of weeds within the Nile basin countries and communities.
- Biotic invasions are serious global problems, which present extreme hazards to the economics and health of natural resource based industries.
- Alien plants have negative effects on biodiversity, agricultural production, livestock, fisheries, and human health. The problem is growing in severity and geographic extent as the volumes of international trade and travel increase.
- The major advantage of biological control is its sustainability. Once an effective natural enemy is introduced and established, regulation of the pest density is perpetual with no additional costs to the beneficiaries.

- In the Nile basin, there are numbers of alien plant species of which water hyacinth *Eichhornia crassipes* the most serious.
- The species is a serious pest in almost all the Nile basin countries.
- In the Sudan alone, the water loss through evaporation and transpiration due to the plant is estimated at 7.1 milliard cubic meters annually.
- The amount of water loss is almost double the amount that can be gained from the construction of Jonglei canal (about 4 milliard cubic meters). This amount can secure irrigation of over 2000 feddans of various crops and secure providing more water to Egypt.

Justification and rational of the project

- Several exotic plants have been purposely or accidentally introduced into Africa. Some of these plants have become serious invasive weeds in agriculture, pasturelands and in waterways; the most important invasive species are *Prosopis spp., Parthenium hysterophorus and Eichhornia crassipes.*
- *Eichhornia crassipes* is the most damaging since it threatens the livelihood of millions of people along the Nile basin. Such threat is not acceptable at all.
- In the Sudan, the weed was declared a national pest in 1959. In Egypt, it was recognized as a serious threat since 1965 after the construction of the High Dam.

- Classical control strategies (Chemical, mechanical and legislative) were futile and costly.
- Classical biological control rests on the premises that organisms are regulated by complexes of co-evolved natural enemies in their native homes. When these organisms (in this case plants) are intentionally or accidentally moved to a new area of the world, they often reach much higher densities than occur in the aboriginal home, because they have escaped their natural control factors.
- Classical biological control strives to re-establish the balances that occur in the native home, in the area, which has been invaded. This is accomplished through the introduction of host specific natural enemies. The natural enemies most often used in biological control of weeds are herbivorous specific and monophagous insects. Plant diseases have also had limited use.

The main goals of the project are

- Reduce the tremendous loss of water resulting from evaporation and transpiration induced by the WH plants.
- Improve navigation along the Nile basin.
- Improve the capacity of irrigation in the major and minor schemes.
- Improve fish habitat which will reflect on fishing capacity and fish industry.
- Reduce the population density of snail, mosquito and sand flies; the vectors of a number of serious diseases in the region.
- Save the high toll due to conventional control strategies, such as high cost and serious externalities resulting from chemical control.

Narrative summary of plan of works: The activities

- Facilities and Equipment: include: establishment of quarantine unit and renovation of biocontrol unit, construction of Mass Rearing soba, dispensable and non-dispensable equipments.
- Evaluation: Water Hyacinth density & distribution through satellite imagery, Ground surveys, plant phenotypic characters, and comparative data before and after BC.
- Importation of *Orththogalumna terebatis.*
- Training: Technicians, M.Sc, PhD candidates in mass rearing, release and evaluation of Water Hyacinth BC.
- Research: Pathogen associated by Water Hyacinth identification, cross breeding between both species of Water Hyacinth weevils.
- Travel: Personnel from Sudan visit Department of BC in Egypt, Egyptian Scientists to visit Sudan to follow up social aspects.

The executed activities of the project

1. A visit from Egyptian Scientists to Sudan in 3/8/2012.
2. Sign the contract in 20/5/2013.
3. Personnel From Sudan visit Department of BC in Egypt in 15-21/ 6/2013.
4. Satellite image (Spot 6) from Kosti to Aldoim.
5. Training in Remote sensing in IGN in Paris (2 trainers from Sudan).
6. Satellite Image from Kosti to Goda at the boundary of South Sudan (Spot 6).

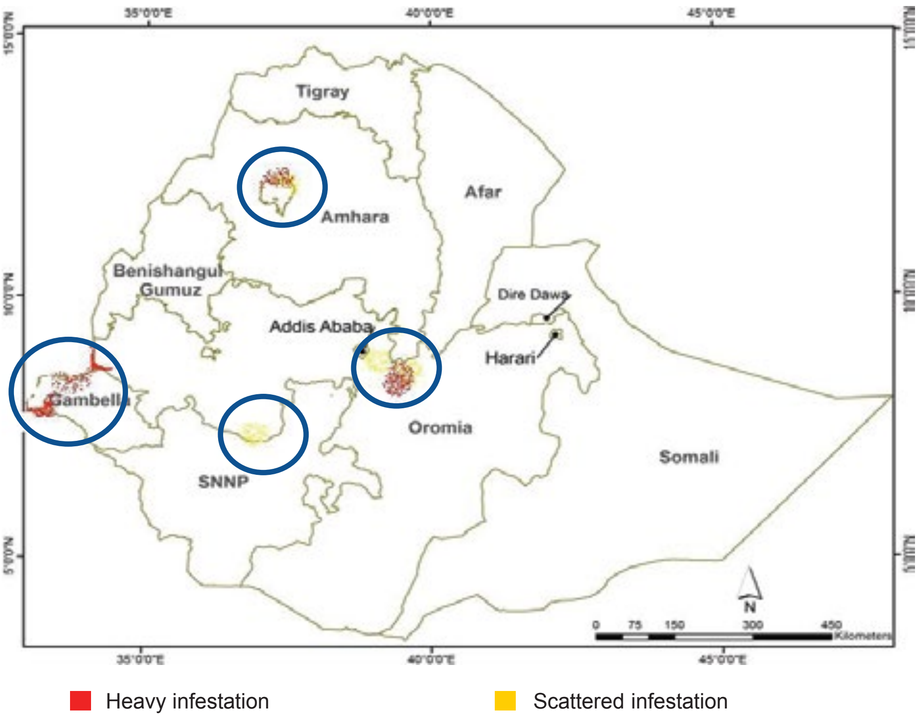
Update status of water hyacinth in Ethiopia: Status and challenges in Upper Blue Nile River

Wassie Anteneh

Introduction

- Invasive alien species are those which have been introduced (intentionally or accidentally) to an area and which cause harm to native biodiversity, human development and human health as well (sometimes) as infrastructure and agriculture.
- While their existence and spread is gradually being realized, capacity and experience to address invasions is limited and the ability and policy environment for rapid response to serious invasive species problems is still limited in most developing countries and Ethiopia is not exception.
- In Ethiopia, close to 45 invasive alien plant species are posing negative impacts on native biodiversity, agricultural lands, range lands, national parks, water ways, lakes, rivers, power dams, road sides, urban green spaces with great economy and social consequences.

- Introduced into Ethiopia (Aba Samuel dam) in 1950s.
- By foreign experts employed at the dam site Ornamental plant.
- The occurrence of water hyacinth in Koka Lake (Awash River) was reported in 1965.
- Baro-Akobo Rivers (Gambella).
- Lake Tana, September 2011.
- Source of infestation.



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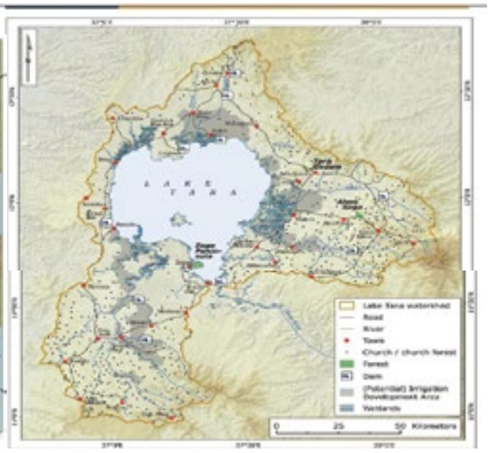
- The largest Lake in Eth (3 2000 km^2).
- Though it is relatively large in surface area, it is shallow (8 and 14 m aver, and max depth respectively).



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- It is internationally important water body:
- Sources of Blue Nile River, major tributary of Nile River.
  - One of the 250 lakes selected for global biodiversity conservation region by Lakenet since it homes 15 unique *Labeobarbus* species (Fish) species flock.
  - IBA site (>300 species).
  - Mammals.

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- Religious and cultural heritage (monasteries and churches).

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- National importance: Growth corridor (huge water resource potential).

HEP: Tana Beles and GERD Fish production Floodplain wtlds (for rice)

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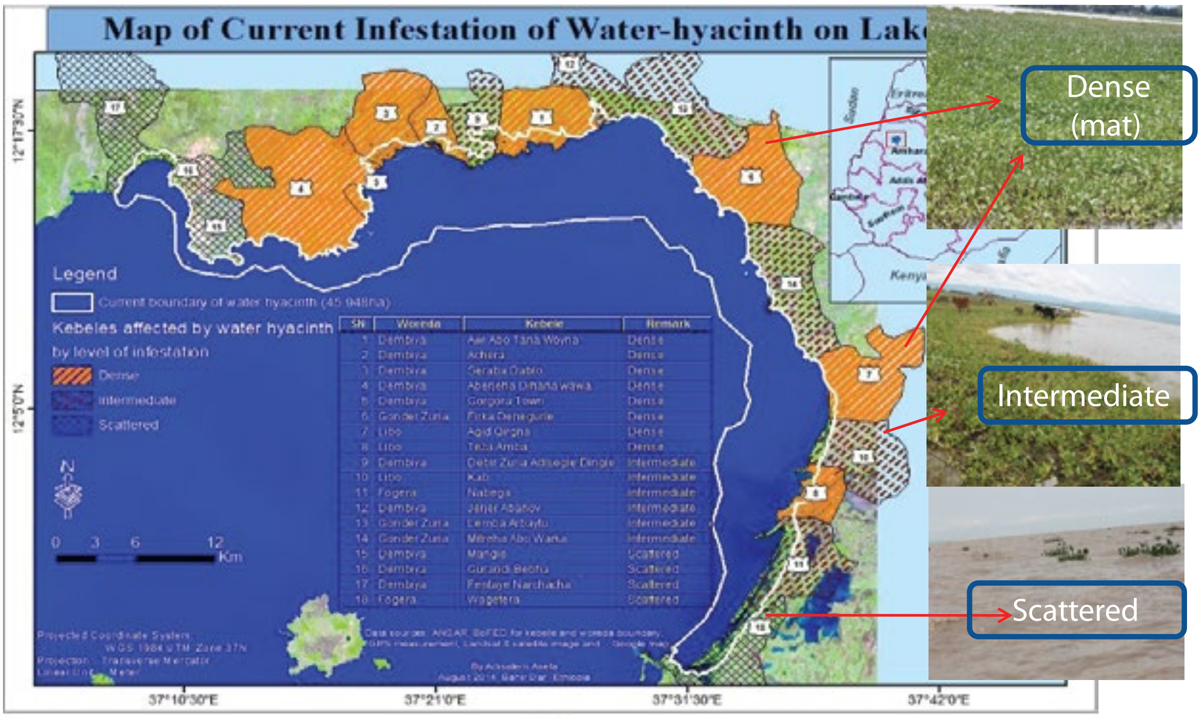
- Biosphere Reserve by UNESCO.
- This multi-purpose lake is facing multi-problems (farming, dams, water removal, pollution etc).
- The final blow for Lake Tana is the recent infestation of water Hyacinth.

2. Status of Water Hyacinth in Lake Tana

- In 2011, it was 4 000 ha.
- In 2012, survey 20 000 ha of the north-eastern shore.
- 40 km shoreline length was infested.
- Distributed in three Districts (Libokemkem, Gondar Zuria and Dembiya) and 15 Kebeles.

- In August 2014 Experts:
  - BDU,
  - ORDA,
  - BoEPLAU and
  - (ARARI) mapped the Water Hyacinth resurgence.

- About 50 000 ha area infestation in 5 Woredas, 128 km Shore length .



SN	Woreda	Kebele	Remark
1	Dembiya	Aar Abo Tana Woyna	Dense
2	Dembiya	Achera	Dense
3	Dembiya	Seraba Dablo	Dense
4	Dembiya	Abenena Dihana wawa	Dense
5	Dembiya	Gorgora Town	Dense
6	Gonder Zuria	Firka Deneguvie	Dense
7	Libo	Agid Qirgna	Dense
8	Libo	Teza Amba	Dense
9	Dembiya	Debir Zuria Adisegie Dingle	Intermediate
10	Libo	Kab	Intermediate
11	Fogera	Nabega	Intermediate
12	Dembiya	Janjer Abanov	Intermediate
13	Gonder Zuria	Lemba Arbaytu	Intermediate
14	Gonder Zuria	Mitreha Abo Warka	Intermediate
15	Dembiya	Mangie	Scattered
16	Dembiya	Gurandi Bebhu	Scattered
17	Dembiya	Fentaye Narchacha	Scattered
18	Fogera	Wagetera	Scattered

- Presented to Agence Nationale de Recherche (ANRS) President and other Bureau heads (August 2014).

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- The Team of Technical Experts:
  - BDU
  - ORDA
  - ARARI
  - BoEPLAU has made the survey in May 2015.
- Result 34 500 ha:
  - Physical removal + Dormancy during dry period.

3. Control Measures

- Water Hyacinth has been effectively controlled from 116 ha of water reservoirs and 15 km of primary and secondary canals of Wonji/Shewa Sugar plantations by draining all the water reservoirs and burning the accumulated dried herbage on the reservoirs floors after glyphosate application.

- The seed bank in the top layer of the soil was removed using heavy machineries. Refilled reservoirs are being monitored to ensure that no infestation occurred.

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- Monitoring in Wonji sugar factory

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Biological Control of Water Hyacinth

- Release of biocontrol agents is also being considered to be used as integral component of the mechanical and chemical control.
- In collaborative activities between Ethiopian Institute of Agricultural Research (EIAR) and the Ethiopian Sugar Corporation Research Directorate at Wonji, two weevils *(Neochetina eichhorniae and N. bruchi)* have been introduced from Uganda by the UNEP/GEF RBIPMA Project of the EIAR and are currently in mass rearing stage in lathouses at Wonji and release of these biocontrol agents is proposed in the nearby neighboring localities of the Koka Lake area after getting approval from the Federal Environmental Protection Authority.
- The pre-release and release plans are not implemented yet due to lack of funding.

- Indigenous fungi found associated with water hyacinth were identified and evaluated for their biocontrol potential against the weed.
- During the study, a series of laboratory and lath house experiments were conducted and 19 fungal species were identified.
- Among these, nine species with better virulence were selected based on preliminary test.
- Host specificity test of potential indigenous biocontrol fungal pathogens of water hyacinth against economically important plants in the Ethiopian Rift Valley.
- Hence, Altenaria altenata and Pythium ultimum were the fungal pathogens which exclusively attacked water hyacinth and could be used for biocontrol agents in integration with insects following their efficacy test under field condition.

4. Control Measure in Lake Tana

- In June 2012, after the surveying report discussion made by higher Regional officials.
- The Regional Government decided to remove the weed using active participation of the community.
- The Regional Water Hyacinth Steering Committee organized which is chaired by the Regional President.


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Steering committee is also organized

- Zonal level
- Worda level
- Kebele
- Bureau of Environmental Protection Land Administration and Use (BoEPLAU) assigned as organizing and implementing agency.
- Public discussion and consultation carried out in every water hyacinth infested Kebeles, the community agreed to remove the weed.


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- Technical Committee also organized (experts from ANRS various sectors such as Bureau of Agriculture (BOA),ARARI, Amhara Organization for Rehabilitation and Development (ORDA) and Bahir Dar University).
- Training of experts.
- Making status surveys.


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- After consultation with the community, in October 2012 Public campaign organized on voluntary basis in every WH infested kebeles:
- Male 193 230
- Female 19 549
- Total 212 779 man-days participated for the manual removal of the weed.

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- 6 827 Ha. was harvested.
- Successful community based manual removal campaign (>95% of WH removed).
- The labor used to harvest the weed was estimated more than 10 million birr.

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Weed Removal Procedures:

1. Harvest the weed

2. carry the weed to dry land

3. Drying of the weed

4. Burning of the weed (fuel)


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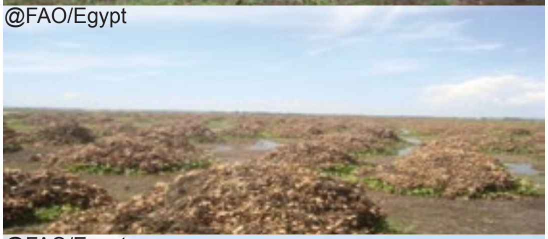
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- As of last MAY, 2015:
- Male 251 307.
- Female 55 646.
- Total 260 992 man-days participated for the removal of the weed.
- 23 487 Ha. Harvested.
- 92% of the weed removed.
- RESURGENCE ~ 34 000 ha.


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Additional Activities

1. National Workshop on the theme "Save Lake Tana and other reservoirs from Water hyacinth" was organized jointly by ANRS, BDU, ORDA and BoEPLAU, (Nov, 28 and 29):

- It Was opened by Regional president.
- Federal state higher officials.
- All Amhara Region Bureau heads.
- Development Partners (FAO, IGAD, Ethiopian Bio diversity Institute (EBI) etc.
- Key experts.


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2. Based on the recommendation from the workshop, WH management unit established by BoEPLAU:
   - Region
   - Woreda level

3.To support the physical removal, team of mechanical engineers from:
   - Bahir Dar University
   - WaliyaPlc
   - ARARI
   - Others from TVT colleges
   - Have designed mechanical tools from local materials


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4. Lake Tana Water Hyacinth Management Strategy developed by a consultant (FAO financed).
5. Organizing Landless youth in WH infested Kebeles (132 groups, 2 263 members). organized groups will be responsible to control the weed in to day to day basis. Thy have the right to use water hyacinth infested land for off farm activities. There will be Continuous controlling of the weed (campaign is not effective).
   strengthening organized landless youth to control the weed To improve their livelihood through different income generating activities.
   Organizing fisher man for continuous scouting purpose (18 groups 1 972 members)
6. Manual Water Hyacinth removal guidelines developed (BoEPLAU).
7. Scout group establishment in non-infested riparian kebeles (54 kebeles).

5. Challenges and Potential Expansions

1. Motivation of local people is declining.
2. Poor International and National Support.
   - Lack of expertise to use other control methods.
   - Poor infrastructure (road and landing).
   - Shortage of logistic (boats, vehicle etc.).
3. High nutrient enrichment from agricultural land and pollution from the emerging cities Bahir Dar and Gondar.

4. Grasses for cattle are destroyed by water hyacinth. Cattle grazing shores are damaged by Water Hyacinth.

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5. Hippo grass is replaced by water hyacinth and hippos have become very aggressive.


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6. Research findings (Tadilo *et al.*, 2014) confirmed Water Hyacinth adversely affected fish breeding and nursery habitats.


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7. Blocking access for water and boat transport.


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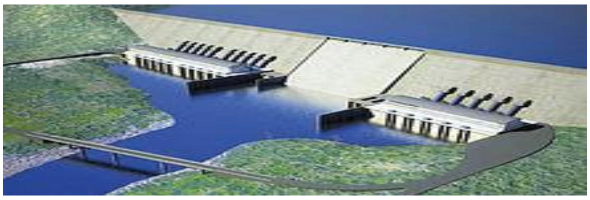
8. May expand to:

A. Tana Beles Power Tunnel (460 MW)

B. New irrigation reservoirs: Ribb, Megech, Koga,Tekeze.

C. Fogera and Dembiya wetlands (Flood plain rice farms).

D. Southern Gulf of Lake Tana (Bahir Dar City and GERD).



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6. Future Focuses

1. Strengthening WH advisory and Research Team (BDU):
   1. Wassie A. (PhD)................Aquatic Ecologist (Team leader).
   2. Ayalew W. (PhD)..............Limnologist (Member).
   3. Melaku W. (PhD)............ Entomologist (member).
   4. Getachew F. (PhD)...........Soil scientist (Member).
   5. Minwuyelet M. (PhD)..... Fish biologist (Member).
   6. Solomon L. (PhD)..............Chemist.
   7. Agegnehu A. (MSc).............Chemist.
   8. Goraw G. (MSc)....................Aquatic Microbiologist .
   9. Yonas (Engineer)..................Mechanical Engineer.
2. Strengthening institutional collaborations (Universities, Research institutions and others):
   - BDU – UoG – DTU platform is already established.
   - University platform and other institutions such (ARARI, EIAR, ORDA etc).

3. Make Use of other WH control methods:

- Mechanical
  - (Uganda)
- Biological
  - (Uganda)
- Chemical

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Neochetina bruchi (left) and *N. eichhorniae* (right) adults

4. Expanding Papyrus Rehabilitation on the shores of Lake Tana.

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5. Research Facilities.
6. Experience sharing, technology adoption and training.
7. PhD and MSc students.
8. International & national partnership and resource.

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Update status of water hyacinth infestation, distribution, impact and management strategies in Lake Victoria, Kenya

Reuben Omondi

Lake Victoria invasion

- Water hyacinth (*Eichhornia crassipes*) was first sighted in the Kenyan water of Lake Victoria in June 1991.
- The weed then invaded Small Water Bodies (SWBs) in the basin.

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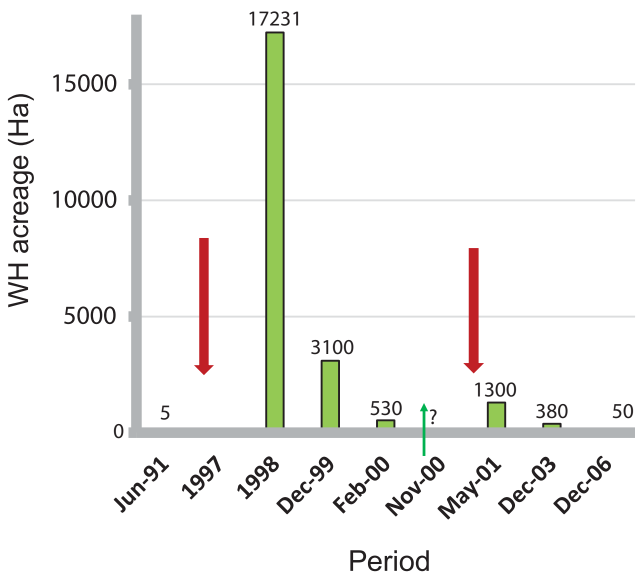
- By 1998, the weed had covered an area of approximately 17 231 hectares, with most found in the Nyanza gulf.
- *Neochetina* weevils were imported in 1997, mass reared and released into the lake.
- The coverage in December 1999 was 3100 Ha which further reduced to 530 Ha in February 2000.
- In 2000 mechanical harvester was used to control the weed in Kisumu Bay.
- After this there have been increase and decrease in acreage of the weed in the lake.



Decrease has been attributed to:

1. Biological agents resilience.
2. Macrophyte succession.

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Impacts Of Water Hyacinth

1. Hydro power production.
2. Disrupts water intake for irrigation and city/municipal consumption.
3. Increase water loss through evapotranspiration (habitat loss).
4. Threaten the ecological integrity of aquatic environment.

Especially Dissolved oxygen and its effect on Fish Distribution


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5. Water hyacinth impacts negatively on fisheries, Water transport, water extraction etc.


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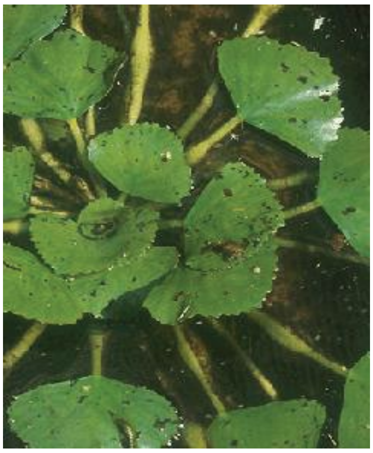
6. Displacement of native aquatic plant species in Lake Victoria.

Azolla nilotica

Pistia stratiotes

Trapa natans



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WH Management project

The Lake Victoria Environmental Management Program (LVEMP)

Phase I: 1997 – 2002

- Funded by: the International Development Association (IDA) and the Global Environment Facility (GEF) at a total cost of US$70 million.
- Overall Objective: Rehabilitation of the lake's ecosystem for the benefit of the 30 million people who live in the catchment, their national economies and the global community.
- WH Specific: Management and control of water hyacinth infestation.
- Notable achievement: Successful water hyacinth control using biological agents through training of personnel, importation of agents and successful multiplication and inoculation.

LVEMP II (2009 – 2017)

- Funded by: The World Bank through International Development Association (IDA) In Kenya the project is funded through the Ministry of Environment, Water and Natural Resources, to the tune of US$30 Million.
- Overall Objective: to have a prosperous population living in a healthy and sustainably managed environment providing equitable opportunities and benefits.
- (WH) specifically to address: The resurgence of Water Hyacinth and other invasive weeds.

LVEMP II WH ACTIVITIES

- Revival of weevils multiplication centres.
- Procurement of mechanical harvester for use in hotspot areas.
- Establish a national water hyacinth Surveillance and Monitoring unit.
- Training of communities on surveillance and monitoring of water hyacinth and biological control methods.
- Carrying out of periodic surveillance and monitoring of water hyacinth and other aquatic plants.

LVEMP II Management

- LVEMP II engaged a National Project Coordination Team (NPCT) headed by a National Project Coordinator and includes technical specialists representing:
  - Water
  - Fisheries
  - Agriculture
  - Forestry
  - Environment - sectors.
- WH placed under agriculture (KARI and KMFRI).
- Core personnel trained under LVEMP I, especially on biological control, had decamped to other sectors (need to train new personnel not heeded to).

WH activities "Score card" based on expected activities

Revival of weevils multiplication centres, Procurement of mechanical harvester for use in hotspot areas, Establish a national water hyacinth Surveillance and Monitoring unit, Training of communities on surveillance and monitoring of water hyacinth and biological control methods and Carrying out of periodic surveillance and monitoring of water hyacinth and other aquatic plants.

1. Revival of weevils multiplication centres

- Multiplication centers established under LVEMP I are not operational.
- Prompted PS to send a delegation to learn from TZ in December 2014. Team visited Mwanza and Musoma.
- No weevil has been introduced into the lake under LVEMP II (From 2006 to date) resilence.


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2. Procurement of mechanical harvester for use in hotspot areas.

- Harvester have not been procured.

3. Establish a national water hyacinth Surveillance and Monitoring unit

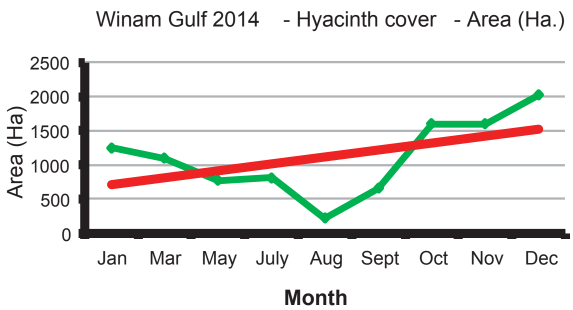
- The unit has not been established despite recommendations following a stakeholders' workshop.

4. Training of communities on surveillance and monitoring of water hyacinth and biological control methods.

- Carried out partially.

5. Carrying out of periodic surveillance and monitoring of water hyacinth.

- Organized stakeholders' workshop.
- Carried out infrequently.
- Reported on the distribution and acreage.
- Trained personnel on use of GIS and remote sensing to determine distribution and acreage. Equipment, however, yet to be procured.



Shortcoming:-
possible underestimation of acreage

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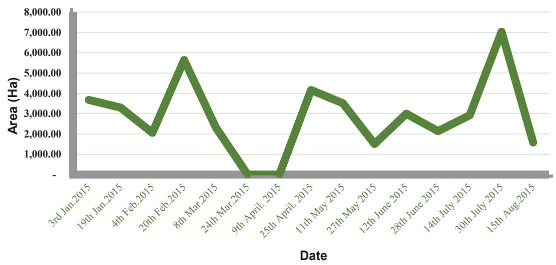

Other activities

Distribution of other aquatic plants in LV and SWBs

- *Egeria* sp recorded around Usenge since 2008 (increasing coverage).
- Other invasive candidates identified in SWBs; *Ceratophyllum, Potamogeton* e.t.c.


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Recommendations

(i) Need for more research on biological control of WH in Kenya (studies can be carried out in the manageable SWBs).

(ii) Need to restructure LVEMP activities by involving technical personnel in their undertakings.

(iii) Need for cooperation between countries sharing same water resources.

(iv) Continuous surveillance and monitoring of aquatic weeds in Lakes, rivers and SWBs in the region.

(v) Urgent need for establishment of national surveillance and monitoring unit.

Acknowledgements

- I am grateful for the invitation to the workshop and to the entire Mansoura University fraternity for their hospitality.

Update Status Of Water Hyacinth Management in Tanzania

Msami Elibariki

Programme main activities

- To conduct surveys to determine distribution, abundance, and damage severity of invasive pests.
- To identify suitable natural enemies through literature search and laboratory studies.
- To import, mass rear and release of effective natural enemies in pest infested areas.
- To conduct post release monitoring surveys to evaluate efficacy of introduced bio agent.
- To conduct training/Awareness creation on the biological control of the invasive pest, diseases and weeds.
- To research on other management options compatible to Biocontrol which can enhance the performance of introduced bio agents.
- To coordinate Biocontrol research conducted in the country.

Status of water hyacinth in Tanzania

- Water hyacinth, *Eichhornia crassipes* (Mart) is native to South America (Center *et al.,* 1999).
- It was first observed in Tanzania in 1955 in the river Sigi and river Pangani in 1959.
- In 1987, the weed had spread to river Kagera which flows to Lake Victoria.
- In 1988, was first observed in lake Victoria.
- In 1995, about 700 ha of water had been covered by Water Hyacinth.

Mat of water hyacinth in lake Victoria

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- In 1998, the coverage in the lake victoria was estimated at 2000 ha (LVEMP report 2000).
- The lake has total surface area of 68 800 sq.km.
- Tanzania shares water of the lake by about 51%.
- Shoreline is about 3450 km long whereby 33% is in Tanzania.

Main Detrimental Effects Of Water Hyacinth

- Reduction in fish in the lake through deoxygenation of water and reduction of nutrients in sheltered bays.
- Physical interference with fishing operations, commercial transportation services for people and goods on the lake.
- Physical interference with access to water supply from the lake, and the cost of purifying water.
- Threats to water intakes at hydroelectric power station in Pangani river.
- Provided breeding habitat for the alternative host for Schistosomiasis (bilharzia) and increased vector mosquito for malaria.

Management Options

- Mechanical use of specialized machines.
- Manual use of simple hand tools.
- Chemicals use of herbicides (Glyphosate and 2,4-D).
- Biological control agents use of weevils (*Neochetina eichhornia* and *N. bruchi*).

Use Of Biocontrol Agents

- In May 1995, a colony of 310 *N. eichhorniae* and 240 of *N. bruchi* was shipped from IITA Benin station in West africa to NBCP-Kibaha Tanzania.
- By Sept 1997, more than 300 000 weevils had been reared and released in river Pangani, Sigi and lake victoria.
- To speed up weevil multiplication, 14 rearing units were established along lake regions (Kagera, Mwanza, and Mara).
- Simtank rearing method was used.
- In year 2000, over 12 million weevils had been produced and released in 80 sites (40 Mwanza, 15 Mara and 35 Kagera regions) lake Victoria.

Weevils *Neochetina eichorhonia* and *N. bruch*

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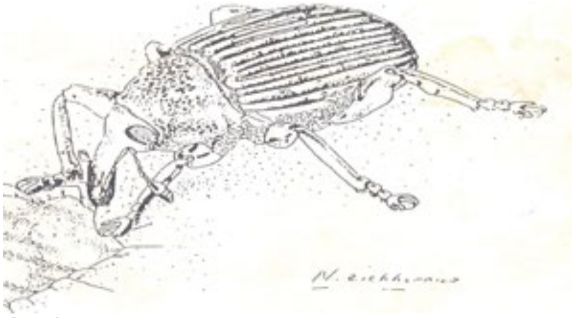

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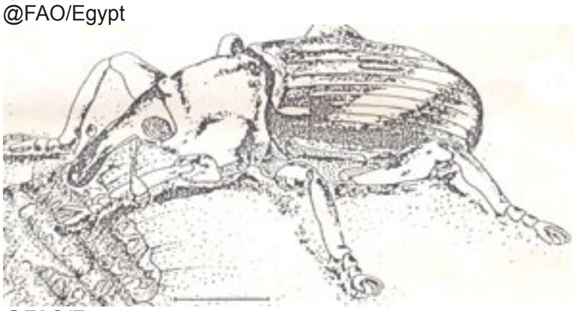

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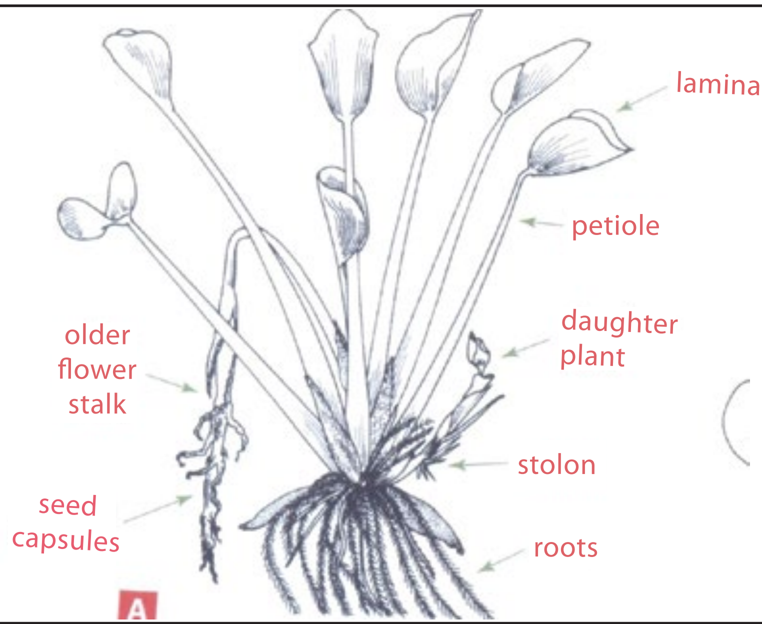

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- Survey conducted in 2004 indicated the weed reduction in plant population from 45 to 7 plants/0.5 m^2, adult populations of up to 30 adults per plant) and and overall reduction by over 80% (Mallya *et al.,* 2004).
- However survey conducted in year 2007 revealed increase in weed cover in lake bays and shores.

Reasons for increased WH

- The resurgence of WH which was attributed to low weevil populations and nutrient loading (Nitrogen and phosphorus from industrial, domestic and agricultural effluents.
- Flushing of fresh hyacinth from upstream of the Kagera river originating from Rwanda draining into the lake (0.2-0.8 ha/day).

National authorities involved in the management of WH

- National biological Control Programme
  - Importation of biocontrol agents in the country.
  - Mass rearing of imported bioagents and release in WH infested areas (rivers and lakes).
  - Monitoring establishment and spread of released bioagents.
- In 1997 Lake Victoria Environment Management Project (LVEMP phase 1funded by World Bank) was initiated.
  - Project carried the task of mass rearing weevils and release in main infested areas in lake victoria.
  - 14 sub rearing units were established along the lake Tanzania side.
  - Manual removal at strategic sites landing beaches and water points to make them accessible.
- Intensification of biocontrol in river kagera, bays and gulfs in lake victoria and in satelite ponds/lakes.
- Community involvement in both manual and biocontrol of WH.
- Capacity building at community and national level in aquatic weed.
- Monitoring and evaluation of WH and impact of control.

Strength and weakness of management practices

Strength

- Periodic surveys and reports for WH infestation along the lake.
- Establishment of enough bioagent sub rearing units along the lake.
- Involvement of communities and NGOs in biocontrol activities, manual removal and monitoring weed infestation and impact.
- Adequate working materials manual work and bioagent rearing.
- Use of telecommunication radios to facilitate communication of weed build up in river kagera, bays and gulfs.

- Aerial surveys and photographs of weed infestation To assess reduction or increase of weed cover.
- Study tour of participants (communities, project staff and NGOs) to other areas.
- Awareness creation through media, posters and booklets.
  - Capacity building to project staff through:
    - workshops
    - short courses
    - long courses

Weaknesses

- Inadequate weevil rearing sub units along Kagera river.
- Rwanda and Burundi was not involved in LVEMP Project Phase I.
- Unavailability of adequate control measures to reduce WH influx caused by Kagera river.
- The status of water hyacinth infestation in the inland waters of Rwanda and Burundi was not clearly documented (Lowe McConnel *et al.,* 1992).

Lessons learnt from LVEMP-I

- Need for a lake wide perspective in tackling environmental degradation of the Lake Victoria.
- There was need for communication of research findings and pilot results to reach policy makers, managers, and stakeholders in language that they understand.
- Involvement of communities in the watershed management was a prerequisite to successful control of non point sources of pollution.
- Private sector has to be involved in a big way for sustainable development of the Lake Victoria Basin.

LVEMPII

- **Objectives:**

(i) Improvement of collaborative management of the transboundary natural resources of LVB for the shared benefits of the EAC Partner States (Including Rwanda and Burundi).

(ii) Reduce environmental stress in targeted pollution hotspots and selected degraded sub-catchments to improve the livelihoods of communities (SIMIYU for Tanzania).

Costructed dam in Simiyu river catchment

- Construction and rehabilitation of the dams at Bubinza and Mwamkala reduced distance to fetch water for domestic use and livestock.
- Contributed to community adoption of improved sanitation and hygiene practices resulting to improving health standards.
- Soil and water conservation project, contributed to improving food security and increased income for the community members.

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Bee keeping in magu district

- Encouraged to grow trees.
- Used the same trees to keep bee hives.
- Group members are owning the project instead of participating.
- Members handle and administer financial resources.

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Biological control of weeds by using plant pathogens

Yasser Shabana

What is the Definition of Biological Control?

Biological control is the use of natural or modified organisms, genes, or gene products to reduce the effects of undesirable organisms (pests) and to favor desirable organisms such as crops, animals, and beneficial insects and microorganisms.

What is the Biological Control Agent?

Biological control agent is any living organism that can be manipulated by man for pest control purposes.

What are the Pros and Cons of biological control (in a general sense)?

Pros

- Host specific
- Nonpolluting
- May provide long-term benefits
- Cost may not be recurrent
- Less costly to develop than chemical pesticides
- Low human and nontarget effects

Cons

- Host specific
- Success unpredictable (performance subject to environmental and ecological factors)
- Slow acting may not satisfy user’s needs
- Sometimes short shelf life

A Comparison between Chemical Pesticides, Biopesticides, and Bioherbicides

Feature	Chemical	Biopesticides	Bioherbicides
Number of agents tested	250	5 500	250
Success ratio	1 in 30 000	1 in 20	1 in 16
Cost of development per agent	US$ 200 million	US$ 2 million	US$ 2 million
Development time	10 years	10 years	10 years
Useful life of the benefit	2.5 - 5 years	30 years	10 - 25 years
Risk of resistance build up	High	Low	Low
Host - target specificity	Small	Large	Large

Modes of Action

The biocontrol agent may control the weed by:

- Producing high levels of disease (pathogenicity).
- Production of phytotoxic and/or enzymatic tissue damage.
- Both ways .

A Flowchart of the discovery and development process for bioherbicides

1. Survey for potential pathogens and collect disease specimens .
2. Isolate, identify, and confirm pathogenicity of potential agents.
3. Determine biocontrol efficacy and safety to nontarget plats (host range) in greenhouse trials.
4. Determin the epidemiological requirements for biocontrol efficacy in the controlled experiment.
5. Develop methods for large-scale production of inoculums for field trials.
6. Confirm biocontrol efficacy in mimnipolt trials.
7. Apply for patents on the bioherbicidal use of promising agents confirm biocontrol efficacy in growers' fields.
8. Seek extramural support (through an industrial partner or Small Business Administration grants.
9. Seek a potential registrant (could be an individual, industry, or Third Party Rregistration, Inc.).
10. Develop efficacy data.
11. Simultaneously, develp toxicology data.
12. Develop industrial process to produce a bioherbicide formulation.
13. Develop a product label.
14. Register the agent as a bioherbicide.

The Future for Biological Control

The most notable changes in the weed control options are:

- The cancellation of the use of methyl bromide as a general purpose soil fumigant.
- The phasing out of several older herbicides during the re-registration process that is underway in the United States.
- The high cost of developing the registering new chemical herbicides.
- The lack of registered herbicides for small markets (minor use crops).
- The emergence of the herbicide-resistant weeds.
- The government instituted mandates for reducing chemical pesticide usage.
- Public preference for the nonchemical alternatives.

All these, in our estimation, will have a profound impact on weed control practices. We expect this emerging situation to create a renewed interest and demand for biological controls.

Status of Research and Development of Pathongens for Biological Control of Water Hyacinth					
Pathogen	Efficacy Tested in		Host Range Tested and Found To Be Safe	More Reasrch Needed on	Practical Use Possible
	Field	Greenhouse			
Acremonium zonatum	No	Yes	Yes	Yes: Field efficacy	Yes
Alternaria alternate	No	Yes	Yes	May not be needed	Doubtful: Efficacy
Aleternaria eichhorniae	Minipolts	Yes	Yes	Yes: Field efficacy	Yes
Cercospora piaropi	No	No	Not tested	Yes: Field efficacy	Yes
Cercospora rodmanni	Yes	Yes	Yes	Yes: Formulation and efficacy	Yes
Helminthospor ium/ Bipolaris spp.	No	Yes	Not tested	Yes: All aspects	Doubtful: Safety
Myrothecium roridum	No	No	Not tested	Yes: All aspects	Doubtful: Safety
Uredo eichhorniae	Yes	Yes	Yes	Yes: Life cycle	Yes
Bacteria	No	No	Not tested	Yes: All aspects	UnKown

Formulation of Microbial Biological Control Agents

- **What is the meaning of "FORMULATION"?**
- Like most chemical herbicides, the active ingredient is combined with inert ingredients in order to make storage, delivery and dispersal convenient and effective. This also allows the user to spread a small amount of active ingredient over a large area.

- The additives have to be inexpensive enough to be economically feasible and should help to enhance the activity of the agent and increase the shelf life (long term viability) of the organism. Also, these additives should help in avoiding the requirements for an extended dew period and the need for high levels of inoculum in the field.

Types of Formulations

1. Liquid Formulations:

- Water is the most commonly used carrier for biological control organisms.
- Surfactants (wetting agents/spreading agents) may be added to break the surface tension of the water.
- Stickers may be added to prevent the wash out of the bioherbicide.
- Humectants may be added to provide an extended dew period (decrease the need for dew), i.e., hydrophilic gels (P syllium mucilloid, cellulose, xanthan gum, gellan gum, commercial polyacrylamide, gelatin).
- These adjuvants may also increase the sticking of the bioherbicide inoculum.
- Oil may be added especially if the bioherbicide spores that are hydrophobic to evenly distribute the spores throughout the inoculum suspension.

Adjuvants used in liquid formulations may increase the efficacy of the organism by:

- increasing the contact of the propagules with the plant surface.
- Prevents the wash out of the inoculum.
- Provide extended dew period.
- Provide nutrients for the biocontrol agent.

In general, the liquid formulations are best for foliar biocontrol agents.

2. Invert Emulsion Formulations (water in oil):

These formulations are semi-solid or thick liquids.

These formulations consist of:

- Aqueous Phase (suspension of the biocontrol agent).
- Oil Phase (mix of mineral oil, vegetable oils, or petroleum products).
- An Emulsifier (i.e., lecithin) to homogenize the two phases.

This type of formulation has allowed for substantial increases in efficacy with dramatic decreases in dew period requirements of many organisms, i.e., *Alternaria eichhorniae, Alternaria cassiae, Colletotrichum truncatum.*

3. Solid Formulations:

Starch encapsulation

- This technique is inexpensive and effective in adhering biocontrol agents to foliage, but requires pre-application wetting.

Wettable Powders

- In this formulation, spore suspensions are slowly dried and then packaged.
- The dried material are easy to transport or storage.

Granules

- This formulation is suitable for use of soil borne pathogens that attack root systems of the target weeds.
- Alginate pellets have been used for formulating many biocontrol agents.

Dust

- Talc, cornmeal, etc. can be used for mixing with the biocontrol agents to produce dust formulations.

Solid Fermentation

- By growing the biocontrol agent on materials such as oats, wheat kernels or bran, and ground corncobs.
- These materials are then allowed to air dry and are applied as a granular composed of the organism + culture medium.

Pesta

- In this formulation, wheat gluten is mixed with the propagules of a biocontrol agent and prepared in sheets (like pasta), the sheets is allowed to dry and then can be crumbled into the required size.

Chapter 2
Technical Papers

Technical Paper on the use of *Neochtina eichhorniae* and *N.bruchi* for biological control of water hyacinth in Egypt

Yahia Hussein Fayad

Preface

This technical paper was prepared under the auspices of the FAO Regional Office for the Near East and North Africa. It aims to help students, scientists, researchers and other interested persons learn more about the floating aquatic weed water hyacinth (*Eichhornia crassipes*), including the problems caused by weed infestations and different methods of control. The paper presents a case study on the biological control of this weed in Egypt using two curculionid weevils: *Neochetina eichhorniae* and *Neochetina bruchi.*

Acknowledgments

The author wishes to express his deep gratitude to the FAO Regional Office for the Near East and North Africa for giving him the opportunity to produce and publish this paper.

Gratitude is also due to both the French Government and the European Union for financing the Biological Control of Water Hyacinth Project since 1999.

A word of thanks and appreciation is also due to USDA for their cooperation and financing of a PL 480 project that supported all preliminary studies for this project from 1978 to 1984.

Introduction

Yahia Hussein Fayad

Water hyacinth, *Eichhornia crassipes* (Mart.) Solms family Pontederiaceae, is the most serious free- floating aquatic weed in the world. The weed was introduced to Egypt during Sultan Mohamad Ali's regime (1805-1848) more than 200 years ago as an ornamental plant, but it was only recorded for the first time by Simpson in 1932. He noticed that the River Nile in Cairo and all Nile Delta governorates were infested with mats of water hyacinth but the southern governorates were all free from infestation. In Egypt – as in many other tropical and subtropical countries – the weed causes acute problems by blocking water canals where irrigation and drainage are economically important, impeding water transportation, disrupting the fishing industry and presenting an environmental hazard. The most important damage done by water hyacinth is the great loss of water caused through evapotranspiration.

The rate of transpiration in a water hyacinth-infested area is about 3.2 to 3.7 times greater than the amount lost from a water-free surface. Based on tests carried out in different parts of the world, it can be concluded that each water hyacinth plant is responsible for the loss of about 0.8 - 1.0 litres of water every day. In Egypt, an investigation was carried out (Khattab and El Gharably, 1982) to determine water losses through evapotranspiration from water hyacinth leaves. The results showed that the actual loss was 1.22 cm^3 / cm^2 /day (based on an average of an entire year). In 1975, Batanouny and El-Fikry reported the presence of water hyacinth in the River Nile. In 1999, the total water hyacinth- infested area in Egypt was estimated at 116 000 feddan (a feddan is equal to 4 200 m^2), covering most of the drainage and irrigation canals, many spot areas on the River Nile and most of the water surface areas of the four northern lakes, which reached about 36 000 feddan. The weed density was estimated at about 240 000 plants per feddan. Accordingly, the amount of water loss through evapotranspiration from water hyacinth leaves was estimated to reach a total amount of more than one billion cubic metres per year. This amount is sufficient to irrigate about 180 000 newly cultivated feddan every year.

It has been stated repeatedly in the literature that evapotranspiration through plant leaves can be an important cause of water loss (Little, 1967; Timer and Weldon, 1967; Mitchell, 1974; Benton *et al.,* 1978). However, the data vary considerably. Estimates of the evapotranspiration from open water surface areas are often above 1.4 and, in some cases, above 3.0. The discrepancies may be related to differences in the applied methodology, but climatic conditions and growth form of the vegetation may also play animportantrole (DeBusk, *et al.,*1983).

In 1965, water hyacinth became a serious problem in Egypt after the construction of the High Dam and flood recession. Manual, mechanical and chemical controls were applied and reapplied as temporary solutions since any unremoved plants regrow quickly after the original plants have been eliminated to repeat the infestation.

Whats is Water Hyacinth?

Classification, Origin and description

1- Classification

The following classification was given by Acevedo and Strong. 2012

Kingdom:	Plantae
Subkingdom:	Viridiplantae
Infrakingdom:	Streptophyta
Super division:	Embryophyta
Division:	Tracheophyta
Subdivision:	Spermatophytina
Class:	Magnoliopsida
Order:	Commelinales
Family:	Pontederiaceae
Genus:	*Eichhornia*
Species:	*crassipes*
Scientific Name:	*Eichhornia crassipes* (Mart.) Solms
English Name:	Water hyacinth

2- Origin, description, distribution and environmental conditions for water hyacinth

Water hyacinth **(Figure1.a)** is a free-floating aquatic weed. (Khattab and Fayad, 1997a) reported that the weed grows worldwide in fresh static or slow flowing water in tropical and subtropical zones. Martius (1824) provided the first description of water hyacinth, noting that the weed originated in South America and is concentrated in North Brazil and Venezuela.

Origin and description

Bennet (1967) observed that the genus *Eichhornia* belongs to the family Pontederiaceae (Monocotyledons). This family contains 21 species under six genera, all of which have aquatic habitats. The five known species of *Eichhornia* are native to South America and the West Indies. The origin of water hyacinth is believed to be Amazonia, Brazil, with natural spread throughout Brazil and to other Central and South American countries (Penfound and Earle, 1948; Sculthorpe, 1967; Little, 1968; Barrett and Forno, 1982).

Perkins (1972) noted that the plant is characterized by its bulbous petiole, shiny waxy green leaves, mass of roots and attractive lavender flower **(Figure1.b)**. He added that the weed is a free-floating plant, the branchemian tissue of the petioles providing buoyancy. The individual plant reproduces by offsets, and thus the mat can grow until it completely blocks the waterway. Vegetative reproduction is a common form of propagation (Julien *et al.,* 1999 and Julien *et al.,* 2001). The weed flowers during summer. The bisexual flowers are bluish purple with a yellow centre. The flowers can self-fertilize. In Egypt, seeds are not common in a flower stand since the life span of the flower is only about 24-48 hours. Accordingly, the common reproductive form of the weed in Egypt is vegetative. In other countries, seeds are produced in large quantities – up to 300 seeds per capsule – and are long-lived, remaining viable for 5-20 years (Manson and Manson, 1958; Matthews, 1967; Das, 1969; Matthews *et al.,* 1977).

Distribution :

A. In the world

Julien *et al.* (2001) observed that the spread of water hyacinth to new areas commenced in the 1880s with its deliberate introduction into the United States as an attractive pond ornamental. They found that water hyacinth live plants were handed out to visitors at the 1884 New Orleans Cotton Expo. Thereafter, the plants continued to spread around the USA and, eventually, around the world. Center (1994) reported that the weed had been recorded in Egypt, Australia and southern Asia by the 1890s (Gopal and Sharma, 1981), China and the Pacific by the early 1900s (Waterhouse and Norris, 1987), East Africa by the 1930s (Chikwenhere, 1994), West Africa by the 1970s (van Thielen *et al.,* 1994) and is now established throughout tropical and warm temperature regions of the world from Portugal in the north to New Zealand in the south (Holm *et al.,* 1977; Julien *et al.,* 1996).

b. In Egypt

Fayad (1982) surveyed water hyacinth infestations in most governorates of Egypt, including the four northern lakes – Mariout, Edko, Al Borolos and Al Manzala – during the period 1978 to 1982 through a PL480 project funded by USDA. The survey indicated that water hyacinth is widely distributed in the governorates of the Nile Delta and heavily infests the four southern lakes. He observed that the weed was also infesting the water surfaces of irrigation and drainage canals in the governorates of Giza, Fayoum, Beni-Suef, Minya and Assiuot. The survey indicated that the most southern governorates of Egypt – Sohag, Qena and Aswan – were absolutely free from water hyacinth infestation. A later survey conducted during 1997 indicated the extent of water hyacinth infestation in Sohag,Qenaand Aswan (Khattab and Fayad,1997a and b).

Environmental conditions

Various research studies reviewed the preferred and optimal environmental conditions for the growth of water hyacinth. The results indicated that the optimal growth of the weed occurs in eutrophic, still or slow moving fresh water with a pH of seven and a temperature range between 28°C and 30°C with abundant nitrogen phosphorus and potassium (Chadwick and Obeid, 1966; Knipling *et al.,*1970; Reddy *et al.,*1989, 1990, 1991). Good growth can continue at temperatures ranging from 22°C to 35°C and plants will survive frosting (Wright and Purcell, 1995). Plants can tolerate acidic water but cannot survive in salt or brackish water (Penfound and Earle, 1948).

Environmental Impact of Water Hyacinth Infestation

The problems caused by water hyacinth in Egypt are similar to those caused by the weed elsewhere in the world. These common and well-known problems can be summarized as follows:

1. Loss of great amounts of water through evapotranspiration, negatively affecting water flow in rivers, canals and drains and thereby imperiling irrigation schemes and slowing drainage of water **(Figures 2 and 3);**
2. Impedingnavigation,fishingandrecreation **(Figure 4);**
3. Degrading water quality by adding taints and odors and decreasing dissolved oxygen;
4. Acting as a good host for mosquitos and the snails that serve as an intermediate vector for schistomiasis (Bilharzia);
5. Threatening engineering structures such as bridges, wires, machines and devices for controlling and measuring water flow **(Figure 5);**
6. Negatively impacting fisheries and the fishing industry;
7. Affecting the diversity of the aquatic ecosystem by altering the flora and fauna as well as preventing sunlight from reaching underwater plants.

Figure 1a. and b. Water hyacinth
(cited from internet)

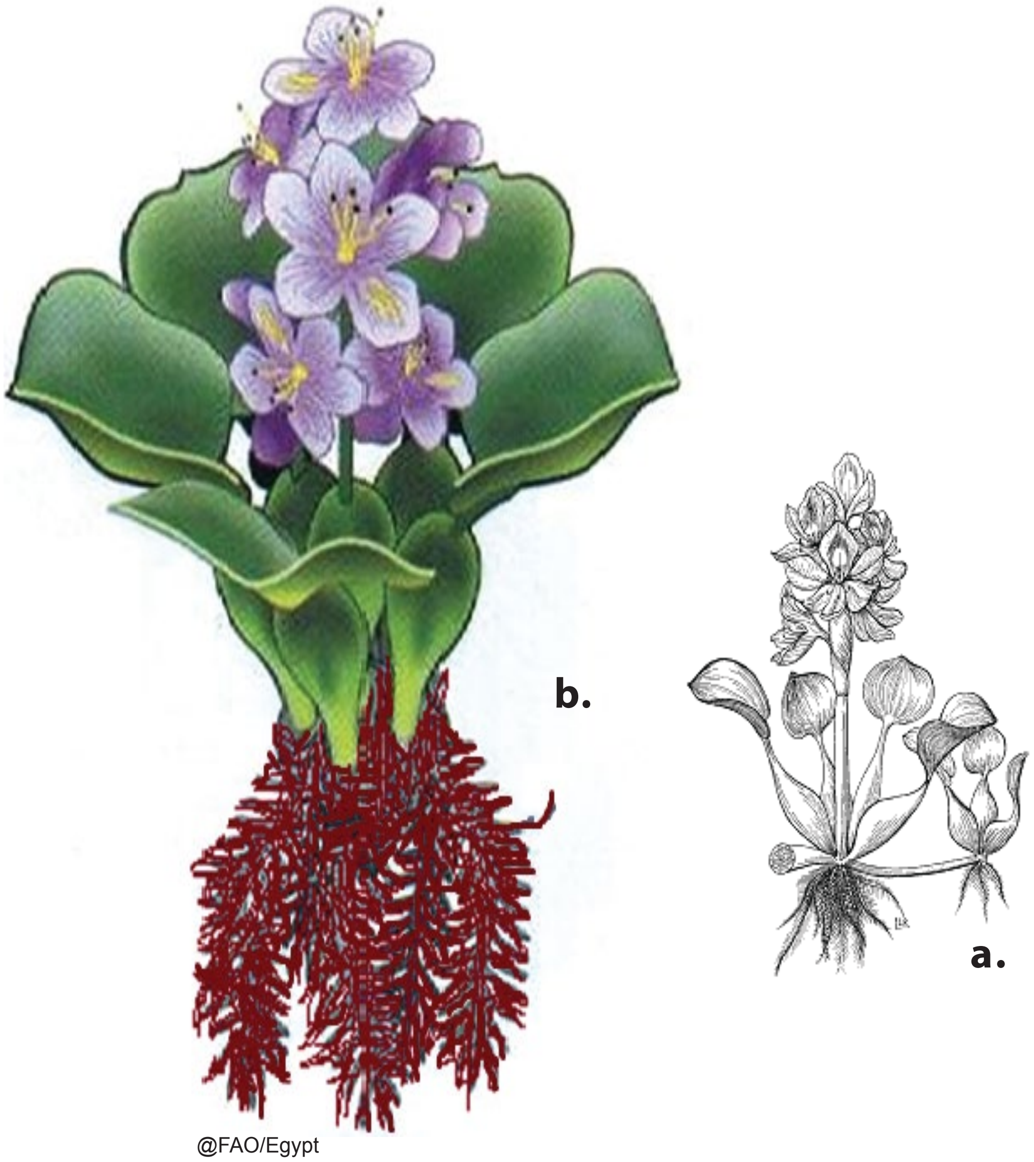

Figure 2. Water hyacinth infestation blocking main irrigation canal in Egypt (1999)

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Figure 3. Water hyacinth infestation in Mariout Lake (Egypt 1979)

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Figure 4. Water hyacinth impedes navigation, fishing and recreation

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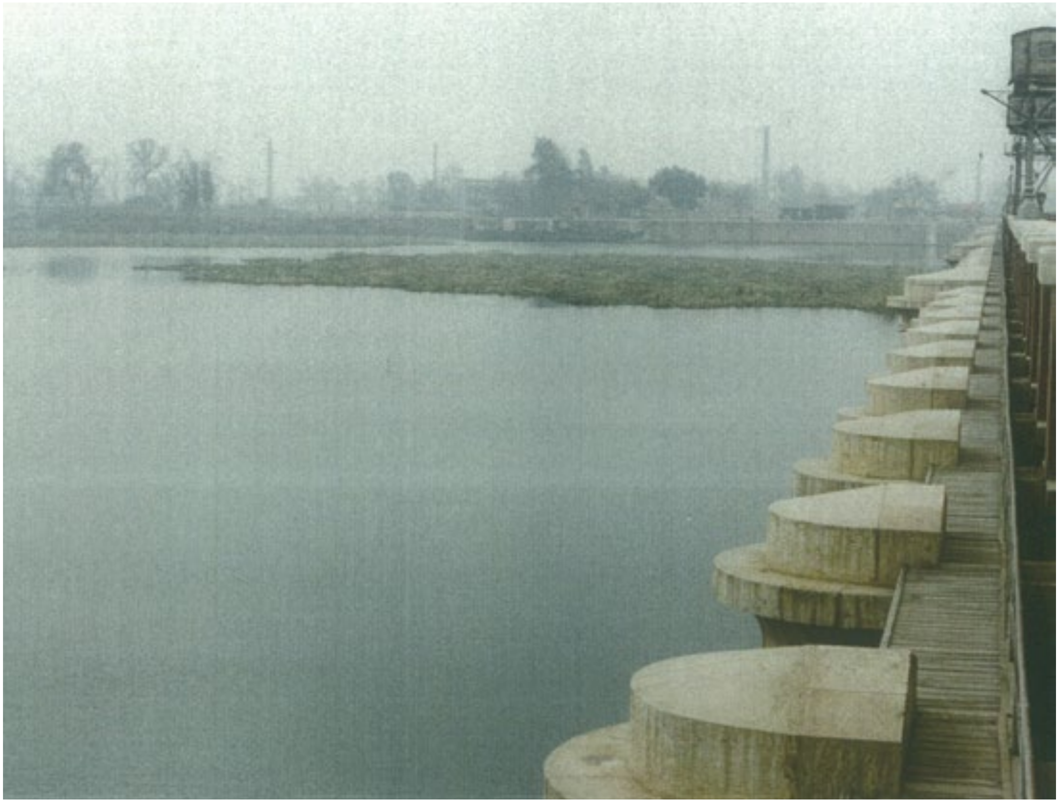
Figure 5. Water hyacinth threatens engineering structures



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Utilization of water hyacinth

Many attempts have been made to use water hyacinth productively. Suggested applications include using the weed as animal fodder, fertilizer, a source of fuel, for the manufacture of paper and handicrafts, and the treatment of waste water and management of water quality. Julien, *et al.* (2001) observed that, despite these ideas, the use of water hyacinth will remain restricted to small- scale industries and is unlikely to provide a viable method for controlling or managing the weed. This is because of the complications and high cost of accessing, harvesting and collecting the weeds, transporting the plants, and drying, processing and marketing the material.

Ninety-five percent of the weight of water hyacinth comes from water (Harley, 1990), making collection costs extremely high for only 5% of dry material. In addition, due to the tendency of water hyacinth to absorb heavy metals from water into the plant tissues, all methods of control should be applied. In developing countries, the small-scale utilization of water hyacinth by local farmers is developing, particularly for the production of biogas as fuel due to the limitation of other energy resources. As a general conclusion, all efforts should be made to dispose of this weed, especially in dry areas where each drop of water is important.

Management of water hyacinth

The irradiation or complete removal of water hyacinth seems to be impossible. Once the weeds are removed, reinfestation occurs quickly. Accordingly, the weed needs to be managed rather than eradicated. Four main methods are known all over the world for the management of water hyacinth. These methods are:

1. **Manual control**
2. **Mechanical control**
3. **Chemical control**
4. **Biological control**

At present, water hyacinth is managed in Egypt by three of these methods since a Presidential Decree was issued in 1990 that bans the use of any chemical on all water surfaces. Accordingly, chemical control is forbidden.

1. Manual control

Khattab and El-Gharably (1982) observed that manual control is the traditional method used for centuries worldwide to manage water hyacinth. They added that hand weeding is, in principle, a very effective way of aquatic weed control. The main advantage of this method is that it is non- selective and all kinds of weeds can be removed. Tools used for hand weeding are a scythe, scythe chain rakes and body powers. Hand collecting in small ditches and small irrigation channels is also common. At present, this method has declined due to the growing recognition of health problems and the risk of getting Bilharzia on the one hand and the significant effort required, with increased labor costs on the other.

2. Mechanical control

Mechanical control is very expensive and slow. It can be applied only in areas less than half a km^2. Weeds are normally removed by cutting or dredging. Rooted submerged weeds are usually cut at a point near the base of the stem leaving the roots and rhizomes undisturbed. The weed will thus regrow and repeat infestation. Dredging removes a portion of the weed buried in the mud from the bottom. It is slower and more costly than cutting. Mainly two types of machines are used for mechanical control: cutting machines and dredging machines.

Floating machines

A number of boats and barges have been designed to cut aquatic weeds.

Weed cutting boats

Mowing boats have been used in Egypt, mainly to open channels through thick mats of water hyacinth.

Harvesters

Harvesters vary in design, but they are basically large barges on which are mounted a belt-type conveyor and suitable cutting machines. The harvested weeds are either collected on the cutting barge or passed to a separate barge and transferred to the shore **(Figure 6a and b)**. In Egypt, three sizes of harvesters of 45 m^3, 25 m^3 and 15 m^3 are used **(Figure 7).**

Machines operating from the bank

Many types of machines are available for controlling water hyacinth from the river bank. Two principal types – excavators and weed cutting buckets – are in use **(Figure 8 and 9).**

Excavators

Excavators can be used to reshape and deepen water channels or to dig new ones. In this case, water hyacinth is removed incidentally. However, in some cases these machines feature a specially- designed rake to remove water hyacinth. In Egypt, the most efficient machine for controlling and removing the thick mats of water hyacinth is a dragline **(Figure 8).** It is used in medium and large channels where satisfactory results can be achieved.

Figure 6a. Harvester used for mechanical control of water hyacinth in Iraq

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Figure 6b. Harvester used for mechanical control of water hyacinth in Iraq

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Figure 7. Harvester used for water hyacinth

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Figure 8. Dragline used for water hyacinth control

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Weed-cutting bucket

In Egypt, side mounted mowing buckets on tractors are in use for removing water hyacinth from channels that are less than three metres wide **(Figure 9a and b).**

Figure 9a. Weed mowing bucket

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Figure 9b. Weed mowing bucket in action

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Other mechanical methods used in Egypt for removing water hyacinth can be seen in **Figures 10 and 11.**

Figure 10. Floating barrier upstream from the Damietta Barrage

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Figure 11. Floating barrier where a harvester of capacity 45 m^3 is collectingwater hyacinth

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Figure 12. Water hyacinth upstream Damietta Barrage

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Figure 13. Removing water hyacinth upstream of a floating barrier by using a motorboat to move the weeds near the bank then removing them using hydraulic excavators

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Figure 14. Floating barrier upstream from the Al Mansoureia Canal

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3. Chemical control

The use of chemical controls on water sources have been prohibited in Egypt since 1990 for the sake of human health and the environment. Although this method of control is very expensive, controlling floating weeds with herbicides is still about half the cost of mechanical methods, according to Khattab and El Gharably (1982). They noted that herbicides were used in Egypt to control water hyacinth from 1975 to 1990. During this period, the most commonly used herbicides were:2.4-D (2.4 dichlorophenoxyacetic acid) from1975-1985; Ametrrryne (Triazine Family) and Reglone (Diquat) (1.1 Ethylene- 2.2 bipytidyldiylium dibromide) from 1985-1990.

The treatment of water hyacinth with herbicides can help to control small infested areas. For example, herbicide was successfully applied to the infested lakes of Mariout, Edko, Al Borolos and Al Manzala. The herbicides most commonly used for water hyacinth are diquat, glyphosate, amitrole and 2,4-D. Chemical controls need frequent reapplication to ensure effective, long-term control of the weed and any regrowth.

4. Biological control of water hyacinth

Water hyacinth is not considered to be a pest in its native habitat, where it is attacked by a complex of arthropods that keep the weed in natural balance. A study of some of these predator species began in Argentina in 1961 as part of a biological control programme (Center, 1994). The study indicated that some of these arthropods cannot survive on any plant other than water hyacinth, while others may only survive on some very closely-related plant species. The first natural enemies of water hyacinth were released in the USA in the early 1970s (Perkins, 1972). By the year 2001, seven agents had been selected as biological control agents for water hyacinth in 31 countries all over the world. These agents are listed in Table 1. (Julien, *et al.,* 2001). The first release of the two curculionid weevils – *Neochetina eichhorniae* and *N. bruche* – took place in Egypt in 2000 (Fayad, *et al.,* 2002).

Table 1: Biological control agents released against water hyacinth worldwide[1]

Agent	Type of damage	Countries where released
Insects: **Coleoptera** **Curculionidae** *Neochetina bruchi* Hustache	Adults feed on foliageand petioles. Larvae tunnel in petioles and crown	Australia, Burkina Faso, Benin, Cote D'Ivoire, Cuba. Ghana, Honduras, India, Indonesia, Kenya, Malawi, Malaysia, Mexico, Mozambique, Nigeria, Panama, Papua New Guinea, People's Rep. China, Philippines, Rep, South Africa, Sudan, Taiwan, Tanzania, Thailand, Uganda, Vietnam, Zimbabwe.
Neochetina eichhorniae Warner	Adults feed on foliage and petioles. Larvae tunnel in petioles and crown	Australia, Burkina Faso, Benin, Cote D'Ivoire, Fiji, Ghana, Honduras, India, Indonesia, Kenya, Malawi, Malaysia, Mexico, Mozambique Myanmar, Nigeria, Papua New Guinea, People's Rep. China,, Philippines, Rep, South Africa, Solomon Islands, Seri lanka, Sudan, Taiwan, Tanzania, Thailand, Uganda, USA, Vietnam, Zambia, Zimbabwe
Lepidoptera **Pyralidae** *Niphograpta* (*Sameodes*) *albiguttalis* Warren *Xubida infusellus* (Walker) (= *Acigona infusella* (Walker)	Larvae tunnel in petioles and buds Larvae tunnel in petioles and buds	Australia, Benin, Ghana, Malawi, Malaysia, Panama, Papua New Guinea, Rep, South Africa, Sudan, Thailand, USA, Zambia, Zimbabwe, Australia, Papua New Guinea, Thailand
Hemiptera **Miridae** *Eccritotarsus catarinensis* (Carvalho)	Adults and nymphssuck cellular or intercellular fluid from leaves.	Malawi, Rep. South Africa, Zambia
Mites **Galumnidae** *Orthogalumna terebrants* Wallwork	Immatures tunnel in laminae	India, Malawi, Rep. South Africa, Zambia
Fungi **Hyphomycetes** *Cerco spora piaropi* Tharp, previously C. *rodmanii* Conway	Punctate spotting and chlorosis of laminae and petioles, necrosis of laminae	Rep. South Africa

[1] Cited from Julien, *et.al.*, 2001.

Fayad (1982) observed that biological control is one of the most important foundations of integrated pest management programmes since it regulates rather than eradicates pest populations. In the case of water hyacinth, any other control methods should be repeated regularly to combat the rapid regrowth of the weed. Harley (1993) noted that the natural enemies that exist in the native habitat of a weed are naturally introduced and established with the intention of reducing the abundance of the weed below the economic threshold; i.e. to a level at which it ceases to be a problem. He added that biological control alone does not eradicate a pest, but aims to establish a natural balance between a weed and its control agents similar to that which occurs in its native habitat.

Biological Control of water hyacinth in Egypt: case study

During the period 1978 to 1984, through a PL480 project and in cooperation with the USDA Agriculture Research Station, Fort Lauderdale, Florida, two curculionid weevil species – *Neochetina eichhorniae* Warner **(Figure 15)** and *N.bruchi* Hustache **(Figure 16)** – were introduced into Egypt under quarantine conditions (Fayad,1982). Both weevil species were studied to confirm their safety as candidates for the biological control of water hyacinth.

Figure 15. *Neochetina eichhorniae*

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Figure 16. *Neochetina bruchi*

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Host specificity tests

Three sets of host preference and feeding tests were conducted.

1. Group plants test:
   All tested plants, including water hyacinth, were exposed simultaneously to *Neochetina* weevils.
2. Paired plantstest:
   Each species of tested plants was introduced, together with water hyacinth, to the weevils.
3. Starvationtest:
   Each of the tested plants was introduced individually to weevils that had been starved for five days. A list of the tested plants and crops can be found in Table 2.

These plants and crops were chosen based on their economic importance to Egyptian agriculture or their natural habitat in relation to their water requirements or the similar structure and morphology of their leaves to water hyacinth.

Table 2: List of plants and crops tested for host preference of *Neochetina eichhorniae* and *N. bruchi* under quarantine conditions in Egypt

Ser. no.	Tested Plants		Family
	English Name	Scientific Name	
1	Banana	*Musa paradisiaca* L.	Musaceae
2	Cabbage	*Brassiea oleracea* L. var. *capitata*	Cruciferae
3	Castor bean	*Recinus communis* L.	Euphorbiacae
4	Taro	*Arum colocasia* L.	Araceae
5	Cotton	*Gossypium barbadense* L.	Mulvaceae
6	Horse bean	*Vicia fabae* L.	Leguminaceae
7	Lettuce	*Lactuca sativa* L.	Compositae
8	Indian shot	*Canna indica* L.	Cannaceae
9	Maize	Zea mays L.	Gramineae
10	Onion	*Allium cepa* L.	Liliaceae
11	Papyrus	*Cyperus papyrus*	Cyperaceae
12	Rice	*Cedrus libani* L.	Conifereae
13	Spinach	*Spinacia oleracea* L.	Chenopodiaceae
14	Sugar beet	*Beta vulgaris var. folloisa*	Chenopodiaceae
15	Vegetable beet	*Beta vulgaris var. rapae*	Chenopodiaceae
16	Water hyacinth	*Eichhornia crassipes* (Mart.) Solms	Pontederiaceae
17	Wheat	*Triticum vulgare Vill*	Gramineae

The results of the tests indicated that the weevils did not feed, deposit eggs or develop on any of the tested plants other than water hyacinth. Accordingly, it was concluded that both *N. eichhorniae* and *N. bruchi* weevils are monophagous insects and safe to introduce as biological control agents for water hyacinth.

Collecting and introducing *Neochetina eichhorniae* and *N. bruchi* to Egypt

During the period 14-21 May, 2000, *Neochetina bruchi* and *N. eichhorniae* were collected from Fort Lauderdale, Florida by handpicking them from water hyacinth plants. The collecting site was chosen due to the occurrence of both species of *Neochetina* **(Figure 17).** The collected weevils were kept in plastic boxes lined with tissue paper and water hyacinth leaves. The containers were transferred to the laboratory, separated by species and examined for pathogenic diseases. Healthy adults were stored in arefrigerator at 10°C.

The collecting procedure continued for five days. A total of 3 004 weevils of both *Neochetina* species were chosen (1 118 *N. eichhorniae* and 1 886 *N. bruchi*). Two to three hundred weevils were placed in screw-capped cylindrical containers (**Figure 18**). The containers were kept in the laboratory at 18°C for two days and then hand carried to Egypt. Upon arrival, the weevils were transferred to a quarantine room attached to the aquatic weed greenhouse at the Department of Biological Control, Plant Protection Research Institute, Agriculture Research Center, Giza, where they were placedina refrigerator at10°C. The following day, the insects were examined and the healthy weevils were released on water hyacinth plants placed in pools under the greenhouse **(Figure 19)**. The greenhouse is 15 x 7 metres with a total area of 105 m^2. It contains nine circular water pools 160 cm in diameter and 100 cm deep each. Each pool holds 1.6 m^3 of water. Water supply and drainage are provided for each pool. The greenhouse is quarantine-guaranteed and includes a dressing room, bathroom and a laboratory.

To ensure the availability of a fresh and healthy water hyacinth supply for the mass production of both *Neochetina* spp., especially during the winter months when water hyacinth is not available in nature, another thermostatically and light-controlled greenhouse was constructed at the research centre in Giza **(Figure 20)**. The greenhouse is 15 m long and 5 m wide and contains two elongated water basins, each 5 m long, 3 m wide and 1.4 m high and designed to hold 13.5 m^3 of water.

Water hyacinth plants were brought in from water canals and the Nile, washed thoroughly and then placed in the two basins. The plants grew well under the greenhouse and quantities of fresh plants were provided to the weevils' rearing greenhouse for feeding *Neochetina* weevils whenever needed.

Figure17. Weevil's collecting site, Fort Lauderdale, Florida, USA, May 2000

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Figure 18. Cylindrical container used to carry the weevils to Egypt

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Figure 19. Water hyacinth greenhouse for the mass production of *Neochetina* weevils

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Figure 20. Greenhouse for growing water hyacinth

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Steps involved in placing water hyacinth and releasing the weevils under the greenhouse

1. Filling the pools with tap water

The pools were filled with tap water, leaving about 20 cm at the top and left untouched for three days to get rid of undesired dissolved gases.

2. Adding fertilizers

Fifteen g of Poly-Feed, 70 g of nitrogen and 10 g of Iron Grapple were added to each pool and stirred. Fertilizer was added every couple of weeks or when needed as the leaves turned yellow or the roots turned blue.

3. Placing water hyacinth plants

Healthy fresh water hyacinth plants were collected from infested irrigation canals and from the River Nile. The plants were cleaned, washed and placed in the pools under the greenhouse. The temperature under the greenhouse was set at an average of 27.8°C. The plants were washed daily with tap water to keep away aphids and mites. Water was added to the pools when needed.

4. Releasing the collected weevils on water hyacinth under the greenhouse

Neochetina bruchi adults were released into six pools and *N. eichhorniae* into three pools **(Figure 21 a. and b.)**. Each pool held 200 - 250 weevils. The rest of the weevils were stored in the refrigerator at 10°C.The pools were examined daily for feeding spots and symptoms caused by the weevils' activities.

5. Establishing insects under the greenhouse

Feeding spots and the weevils' activities, including mating **(Figure 22)** and oviposition, were observed two days after release. Day after day, the damage to the water hyacinth plants became more obvious and new plants were added periodically. The first generation of *Neochetina* adults emerged on 15 August, 2000.

Figure 21a and b. Releasing the introduced weevils under the aquatic weed greenhouse

a.

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b.

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Can releasing *Neochetina* spp. control water hyacinth?

Water hyacinth plants reproduce and multiply by offsets (Perkins, 1972). Fayad *et al.* (2001) noted that water hyacinth plants double in number every eleven days. They added that a pair of water hyacinth weeds can cover a total water surface area of about half of a km^2 within three months. (Fayad, 1999a; Fayad *et al.,* 2004) indicated that both *Neochetina eichhorniae* and *N. bruchi* are monophagous. They can't feed, oviposit or complete their development on any plant other than water hyacinth. They are nocturnal, hiding during the daytime and active at night. The adults feed on the plant leaves and petioles, causing feeding spots with dead decayed tissues that cannot evapotranspirate **(Figure 23)**. The adult females deposit their eggs inside plant tissues **(Figure 24)**. Each female deposits average of 250-350 eggs during her lifespan (Fayad 1982). Hatched larvae feed on internal plant tissues, boring downwards until they reach the plant reproductive area under the water surface and destroy it **(Figures 25 a and b)**. Full grown larvae use decayed tissues and rootlets to form cocoons and pupate inside of them. The cocoons are attached to the weed roots under the water surface **(Figures 26 a and b).**

Figure 22. Mating process of *Neochetina* spp

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Figure 23. Feeding spots of *Neochetina* adults

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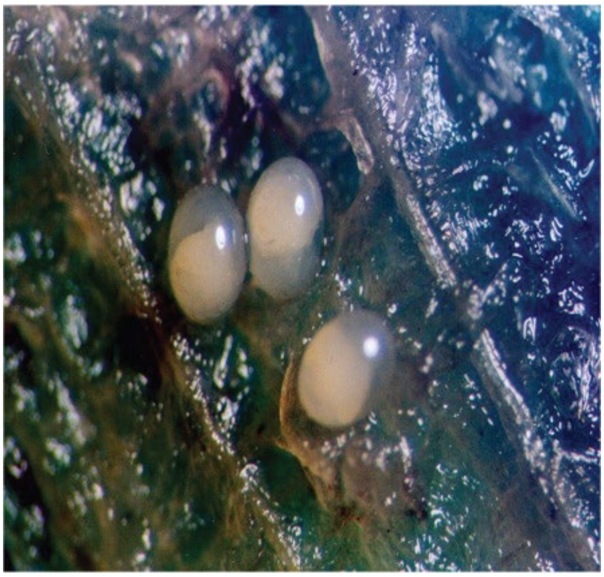
Figure 24a and b. *Neochetina* spp. deposited eggs

a. Newly deposited eggs



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b. Prehatched larva

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Figure 25a and b. *Neochetina* Larval Damage

a.

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b.

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Figure 26a and b. Cocoons of *Neochetina* spp attached to roots of water hyacinth

a.

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b.

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Figure 27a and b. Collecting *Neochetina* adults under the greenhouse

a.

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b.

@FAO/Egypt

Collecting *Neochetina* weevils from the greenhouse and releasing them onto water hyacinth infestations in the four northern lakes of Egypt

Neochetina adults were collected from water hyacinth plants grown in the greenhouse either by hand picking or by using a metal ring to sink the weeds under water for 20 minutes, then collect the floating weevils by means of a strainer **(Figure 27 a and b).** The collected weevils were kept in boxes and stored in incubators for further use at a constant temperature of 10°C. Fayad *et al.* (2002) indicated that the weevils can be stored up to six months and still survive and be active.

Starting in 2000, the weevils were released in two northern lakes: Mariout and Edko. They were released in two additional northern lakes – Al Borolos and Al Manzala – from 2003 to 2006 **(Figure 28)**. The weevils were released early in the morning or before sunset using airboats, rubber motorboats or small fishing boats **(Figure 29)**. The number of weevils released each year can be found on Table3.

Table 3. Annual numbers of released *Neochetina* in the four northern lakes of Egypt

Year	AlManzala	Al Borollos	Edko	Mariout	Total
2000	-	-	4 271	2 302	6 573
2001	-	-	4 750	4 500	9 250
2002	-	-	-	-	-
2003	2 000	-	-	3 750	5 750
2004	3 036	2 750	-	-	5 786
2005	1 500	250	-	250	2 000
2006	3 042	-	-	-	3 042
Total	9 578	3 000	9 021	10 802	32 401

Figure 28. The four northern lakes of Egypt where weevils were released

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Figure 29. Releasing *Neochetina* weevils to control water hyacinth in Egypt’s northernlakes

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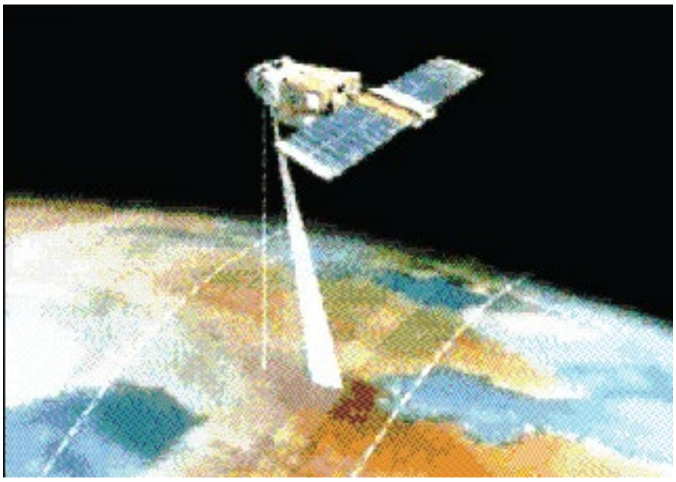
Monitoring and evaluating the effect of releasing *Neochetina* weevils onto water hyacinth infestations in the four northern lakes

Satellite images of the four lakes using the French Spot 5 Earth observation technology were captured before and one year after releasing the weevils onto the lakes (Fayad *et al.,* 2003). The advantages of using the satellite images are summarized in **(Figure30)**.



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- One scene covers an area of 60X60 Km^2.
- Very high resolution.
- Passes over the same point every 26 days.
- Distance between each cycle is 3833 Km.

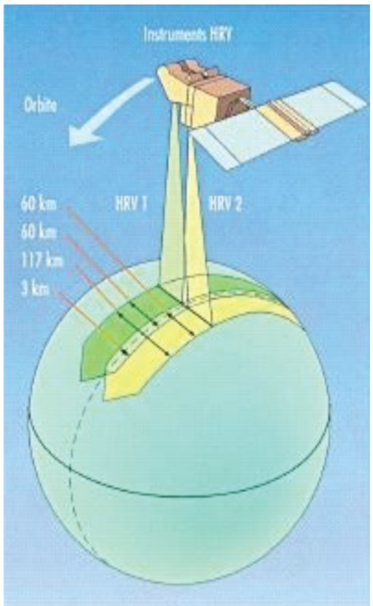

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Figure 30. Advantages of using satellite images

Periodic visits to the release sites on the four lakes were conducted for follow up. Evidence of feeding spots indicated the establishment of *Neochetina* weevils at the sites. Further visits indicated the wide spread of the weevils over all water hyacinth infestations. Results obtained from the satellite images of each lake are shown and summarized in **(Figures 31, 32, 33 and 34).**

Mariout Lake

Figure 31. Satellite Water hyacinth infestation in Mariout lake

a. before release of weevils

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b. after release of weevils

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Total area covered by water hyacinth in 2000: 2 330 km^2
Total area covered by water hyacinth in 2001: 1 652 km^2
Total area of the lake in 2000: 220 619 km^2
Rate of water hyacinth infestation during 2000: 1.06 %
Reduction in water hyacinth infestation due to weevil release 2000 / 2001: 29.1%
Rate of reduction in water hyacinth infestation by 2008: 65%

Edko Lake

Figure 32. Satellite images of Edko Lake

a. before release of weevils

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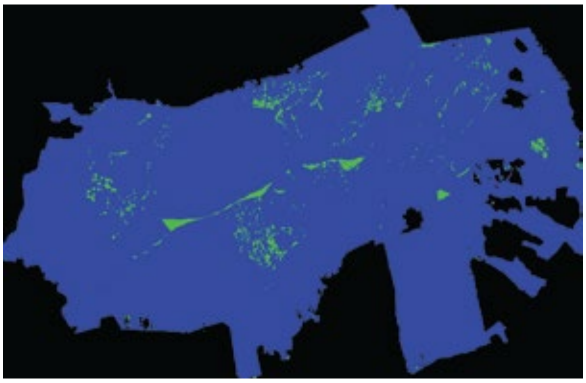
b. after release of weevils



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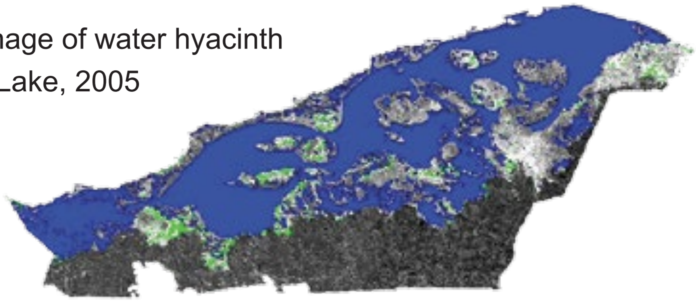
Total area covered by water hyacinth in 2000: 4 105 km^2
Total area covered by water hyacinth in 2001: 2 480 km^2
Rate of water hyacinth infestation in 2000: 5.76 %
Reduction in water hyacinth infestation from 2000 to 2001: 39.6 %
Rate of reduction in water hyacinth infestation by 2008: 96%

Borollos Lake

Figure 33. Satellite image of water hyacinth infestation in Borolos Lake, 2005



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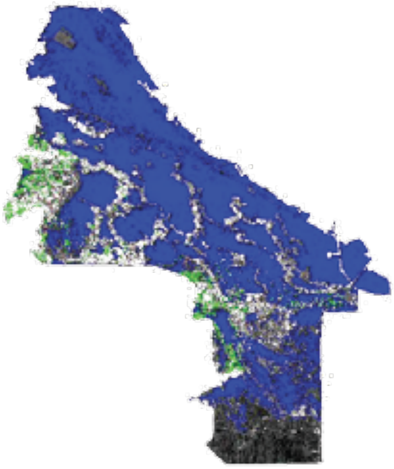
Total area covered by water hyacinth in 2000: 29 929 km^2
Total area covered by water hyacinth in 2005: 11 529 km^2
Reduction in water hyacinth infestation from 2000 to 2005: 44.9 %
Reduction in water hyacinth infestation from 2005 to 2006: 37.04 %
Reduction in water hyacinth infestation from 2000 to 2006: 65.31 %
Estimated reduction in water hyacinth infestation by 2008: 95%

Manzala lake

Figure 34. Satellite image of water hyacinth infestation in Manzala Lake, 2005



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Totala reacovered by water hyacinth in 2000: 33 016 km^2
Total area covered by water hyacinth in 2005: 27 930 km^2
Reduction in water hyacinth infestation from 2000 to 2005: 15.4%
Reduction in water hyacinth infestation from 2000 to 2006: 33.14 %
Reduction in water hyacinth infestation from 2005 to 2006: 20.98 %
Estimated reduction in water hyacinth infestation by 2008: 80%

The control results indicated the widespread establishment of the weevils throughout the country. The weevils were found associated with water hyacinth all over in Egypt, even in areas where they had not been released. In general, the results are sustainable and a level of natural balance has been achieved between the water hyacinth and the weevils. It should be mentioned that the cost of applying weevils as biological control agents is approximately 500 000 Egyptian pounds a year (US$ 28 000) as compared to the cost of mechanical control at 150 000 EGP a year (US$ 8 380).

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Technical Paper on the use of *Alternaria eichhorniae* as a mycoherbicide for water hyacinth

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Abbreviations

Ae5	*Alternaria eichhorniae* isolate# 5 (Egyptian isolate)
RH	Relative humidity
DS	Disease severity
DI	Disease incidence
PDA	Potato dextrose agar medium
PDB	Potato dextrose broth medium
MRB	Modified Richard's broth medium

Introduction

In view of the problems encountered in water hyacinth management, attention has recently focused on biological control. Biological control can provide a lasting, cost-effective and environmentally safe solution, and is theoretically the best method for solving the water hyacinth problem (Shabana *et al.,* 2001b). Recent conferences and panels have thus stressed the need for renewed and greater emphasis on biologically -based alternatives (Delfosse and Spencer, 1997; Charudattan *et al.,* 1996).

Alternaria eichhorniae Nag Raj and Ponnappa, is a fungal pathogen that has been reported to be associated with water hyacinth in Egypt, Kenya, Zimbabwe, Ghana, Uganda, South Africa, India, Indonesia and Thailand. This fungus has shown to be host-specific to water hyacinth and capable of severely damaging and suppressing the weed (Shabana *et al.,* 1995a, b & c). A good understanding of the biology and pathology of this fungus has been gained (Shabana *et al.,* 1994, 1995a, b; 1997a, b; 2000; 2001a, b). The major constraint to its use as a biocontrol agent for water hyacinth – the need for a long period of dew – has been overcome in miniplot field trials by formulating the fungus in oil emulsions (Shabana 1997a, b; Shabana *et al.,* 2001a). The findings of Shabana et al. in 18 years of research (see references) has confirmed the feasibility of using *Alternaria eichhorniae* 5 (isolate Ae5) for the biomanagement of water hyacinth in Egypt.

The objective of this paper is to explain the themes of isolation, identification, pathogenicity testing, host range testing, culturing and mass production, spore production, phytotoxin production, and formulation and application of *A. eichhorniae* as the most promising mycoherbicidal agent for water hyacinth in Africa.

Isolation

Alternaria eichhorniae is associated with severe leaf spotting and blight, which spread rapidly from disease foci (Shabana *et al.,* 1995a). Typical symptoms first appear as necrotic brown flecks that enlarge gradually, developing yellowish chlorotic surrounding haloes and then coalescing into blotches with brownish to dark brown centres and faint yellow margins **(Figures1 and 3).**

Exercise 1

To isolate the causal agent from diseased water hyacinth leaves, follow these steps **(Figure 4):**

1. Wash the diseased leaves carefully in running tap water followed by sterile water.
2. Cut leaves into small pieces (1-2 cm^2) with individual spots in their centres using sterile scissors or blade. Collect the pieces in a sterile Petri dish containing sterile water.
3. Transfer the pieces to a Petri dish containing surface sterilizing solution, i.e., 10-20% Clorox (0.5-1% sodium hypochlorite; commercial bleach contains about 5% sodium hypochlorite) for two to five minutes or 0.1% mercuric chloride for ten minutes and rotate the plate to keep the leaf pieces covered by the solution.
4. Wash thoroughly with sterilized water to rinse out all remaining traces of disinfectant solution.
5. Blot dry the sterilized pieces onto sterile filter papers using forceps.
6. Place the pieces on water agar (WA) or potato dextrose agar (PDA) supplemented with antibiotics (streptomycin sulphate, 0.3 g/l and chloramphenicol, 0.1 g/1). Place four to five pieces on a nine cm diameter plate.
7. Incubate the plates at 25-28°C for two to four days.
8. Separate different cultures (resulting on plated pieces) on different plates containing thin layers of water agar (preferably) or potato dextrose agar plus antibiotics (see step 6).
9. Employ hyphal tip or single spore isolation techniques to obtain pure cultures of fungal isolates.
10. Maintain pure cultures on slants containing potato dextrose agar or special nutrient -poor agar consisting of 1g $KHIO_4$, 1g KNO_3 , 0.Sg $MgSO_4$ x $7H_2O$, 0.5g KCl, 0.2g glucose, 0.2g sucrose, and 20g agar/l (Nirenberg 1976) in refrigerator.

    Soil cultures are recommended for the long-term preservation of *A. eichhorniae.* Sterilize screw-capped tubes, each containing five g of organic-free soil (clay/sand at 1:1) in an autoclave for one hour every 24 hours for three consecutive days. Let the tubes cool down, then add 2.2 ml of fungal mycelial suspension to each tube and incubate at 28°C for 10-15 days. Thereafter, keep the tubes refrigerated.

Identification

The colony growth and morphology of the fungus, its conidial measurements (Table 1), ability to produce pink pigments on PDA or potato dextrose broth media (PDB) **(Figures 5 and 6)** and pathogenicity to water hyacinth **(Figures 1 and 3)** were the criteria used for describing *A. eichhorniae.* The most distinct conidial features of the Egyptian isolate, Ae5, were the total length of conidium, the size of the beak, the proportion of beak size to total conidial length, and the broadest width of the conidial body **(Table 1).** Sometimes the conidia of Ae5 are in short apical chains of three to four and – rarely – five conidia.

Table 1 : Conidial dimensions of *Alternaria eichhorniae* Nag Raj and Ponnappa and *A.eichhorniae* isolate # 5 (Ae5, Egyptian isolate)

Isolate	Total length (µm)	Beak		Broadest width[e] (µm)
		Length[a] (µm)	Proportion[b]	
A. eichhorniae Nag Raj and Ponnappa[c]	21-212 (51) NM[d]	Up to 145 NM	$^{1}/_{3}$ to $^{2}/_{3}$	9-19 1 4
A.eichhorniae isolate # 5 (Ae5, Egyptian isolate)	42-165 (61) 80	11-66 (19) 26	$^{1}/_{4}$ to $^{2}/_{5}$	7-13 (10) 10

[a] Values represent the range, mode (within brackets), and average length .

[b] Length of beak as a fraction of the conidial length.

[c] According to Nag Raj and Ponnappa (1970).

[d] Not mentioned in the reference cited.

[e] Based on measurements of 100 conidia grown on potato dextrose agar.

The growth of Ae5 on PDA is fairly rapid (four - five cm diameters of colony can be grown in six days).The mycelium is aerial, velvety to cottony in the middle, changing color from ‘shell pink’ to ‘vinaceous purple’ and ‘dull Indian purple’ (Ridgway, 1912). The culture is indented slightly at the perimeter with an effuse cottony mycelium rising to a height of six mm. It turned the color of the medium to ‘tourmaline pink’ or ‘Indian lake’ **(Figures 5 and 6).**

Pathogenicity testing

Exercise 2

Preliminary test

1. Grow fungal isolates on PDA for 7-14 days at 28°C in the dark without wrapping plates with Parafilm (you may wrap plates in a single layer, if necessary).
2. Collect healthy-looking, medium-sized (4-6 leaves) water hyacinth plants, wash them with running tap water and prepare them for inoculation as follows: insert clear polyethylene bags (35x 45 cm^2) into pots (1 L size), fill the plastic bags two-thirdswith water, and then insert individual water hyacinth plants into each bag **(Figure 7).**
3. Put two to three agar discs of the fungal culture (upside down) on each water hyacinth leaf and petiole. Spray the internal surface of the plastic bag with water using an atomizer and then fold the top edge of the bag and staple it closed. Keep the bags closed for two days to maintain high relative humidity **(Figure 7).** Make three replicates (plants) per treatment.
4. Control plants are treated similarly but with fungus-free agar discs. It is preferable to begin with control plants to avoid cross contamination.
5. Observe plants for the appearance of disease symptoms around the discs.

Exercise 3

Standard test

1. Grow fungal isolates on PDB (still or with shaking) for 7-14 days at 28°C and in the dark.
2. Prepare water hyacinth plants for inoculation one day before inoculation (as described above) to help plants settle down and adapt.
3. Spray plants with suspensions of conidia (1 x 10^6 per ml) or mycelia (10% weight/volume [w/v]) obtained from the above culture. The latter inoculum is prepared by blending 10 g (wet weight) of mycelium in 100 ml sterile water for six seconds at high speed in a blender. The inoculum is applied alone or with 0.5% w/v Metamucil (psyllium hydrophilic mucilloid) as a humectant. Metamucil is added to the inoculum suspension or to plain water (as a control) and allowed to hydrate for 30 minutes before being sprayed on the plants with a hand-held low-pressure sprayer until runoff occurs. Follow the procedure described above to maintain high relative humidity. Make three replicates (plants) per treatment.
4. Determine the disease severity (DS) for each leaf with the help of a pictorial disease scale of O to 9, where O is healthy and 9 is 90% diseased (Freeman and Charudattan, 1984) **(Figure 8).** Values for individual leaves are the summed and averaged to derive DS for a whole plant.

Host range testing

Plant species and cultivars of economically important crops should be screened (preferably in two consecutive years) against isolates that are shown to have a pathogenic effect on water hyacinth to determine the safety of their use as biocontrol agents.

Host range studies were conducted in small field plots at the University of Mansoura campus in Egypt to determine the safety of Ae5. Pathogenicity of the isolate to 97 economically important terrestrial plant species and cultivars grown in Egypt was determined in two consecutive years (1987/88 and 1988/89), using three replicates (miniplots) per plant species/cultivar (Shabana, 1992; Shabana *et al.,* 1995a). Water hyacinth was used as a positive control. In the first year, mycelial inoculum obtained from three-week-old cultures grown on PDB containing 2.5% yeast extract was used. The inoculum was used as an aqueous suspension of fresh mycelium (8.5% wet w/v) and sprayed with a hand-operated, low-pressure sprayer until runoff occurred. In the second year, the mycelium was entrapped in alginate pellets (Walker and Connick, 1983), dried, ground into a powder (300 to 600 µm), mixed with 1o/o (w/v) polyacrylamide (Evergreen 500), and broadcast over the field plot area until each test plant had been exposed to the inoculum. In both years, the plants were inoculated at the seedling stage and again at the preflowering stage.

Culture conditions, mass production and inoculum efficacy

Results showed that the efficacy of the spore inoculum was equal to that of the mycelial inoculum of Ae5. The disease was initiated with either conidial or mycelial inoculum to yield a similar level of DS (Shabana *et al.,* 1995a). The ability to use mycelial inoculum is important to the development and large-scale field use of Ae5 because themycelial inoculum is easier to produce than conidia.

Optimum media, light regime, incubation temperature, pH and aeration conditions were determined for the culture growth of Ae5. Maximum linear growth was obtained when Ae5 was grown on water hyacinth-fresh potato dextrose agar and on water hyacinth dextrose agar at pH 7 and incubated at 30^{o}C under continuous light on unwrapped plates **(Figures 9 and 12)** (Shabana *et al.,* 2000).

Inexpensive alternatives to the costly laboratory media should be sought. For example, the use of molasses, grain mash, fish meal, soybean meal, whey, cottonseed flour, etc., which are often readily available at low cost and in unlimited quantities, should be investigated (Churchill, 1982).

Nevertheless, the optimum conditions for the production of highly virulent inoculum were obtained when stationary cultures of Ae5 were grown on fresh PDB under diurnal light for one week, followed by continuous darkness for an additional week (Shabana *et al.,* 1995a). In addition, stationary cultures grown on PDB at 28°C in the dark for 10-20 days produced highly virulent inoculum (Shabana, unpublished).

The optimum blending time for preparing the mycelial inoculum of Ae5 for spraying was six seconds. Mycelium blended for that period of time provided the highest disease incidence (DI) and DS (Shabana *et al.,*1995a).

Disease severity increased with increasing inoculum density up to 10% mycelial wet weight; a higher inoculum density did not provide greater DS (Y. M. Shabana, unpublished).

Exposure of inoculated leaves to at least ten hours of dew was conducive to a high level of disease, and the use of a hydrophilic mucilloid as a humectant with the mycelial inoculum augmented the disease level (Shabana *et al.,* 1995a).

Spore Production

Among four methods (modified Walker's, Shahin's, modified Cotty's and the sodium alginate method) and physical factors (temperature, light, pH, aeration and culture media) tested for inducing spore production by Ae5, Walker's method (Walker, 1980) was the best since it required less time and fewer materials, while producing a larger quantity of spores than the other techniques (Shabana, 1992; Shabana *et al.,* 2000).

Exercise 4

Modified Walker's method for the large-scale production of Ae5 spores

1. Grow Ae5 on modified Richard's broth (MRB: 10g sucrose, 10g KNO_3, 5g $KHIO_4$, 2.Sg $MgSO_4.7H_2O$, 0.02g $FeCl_5$, 150 ml V-8 juice and distilled water to make 1 litre and adjust it to pH 6 before autoclaving on a reciprocating shaker (120 strokes/min) at 25 ± 3^{o}C under diurnal light or in continuous darkness for seven to ten days. It was found that still cultures did not produce spores with this method (Shabana, unpublished). But this result needs to be confirmed by repetition.
2. Collect the mycelial mat by filtration through cheesecloth.
3. Blend 10 g (wet weight) of mycelial mat with 10 ml of distilled water and 80 µl of antibiotic solution containing streptomycin sulphate (3.7mg/ml) and chloramphenicol (2.5mg/ml) for 20 seconds at high speed in a blender.
4. Pour the mix into 9 cm Petri plates lined with aluminum foil (20 ml mycelial homogenate per plate).
5. Place the plates in large plastic pans supported over glass stilts, containing tap water and covered with a clear plastic hood to maintain high humidity. Two apertures should be made on the sides to allow ventilation. Place the whole set (the large pans with Petri plates) under two 40 W cool-white or Gro-Lux fluorescent lamps suspended 45 cm above the plates. Start incubation with seven hours of light followed by 13 hours of darkness, and then four hours of light at 30°during the light and 25°C during the dark periods, at high RH (80-90%) for 24 to 48 hours.
6. After 24 to 48 hours, the surface of the mycelial layer will be black to dark olive green and covered with conidiophores and conidia. Turn the light off for 24 hours and maintain RH at 90-98% to allow the spores to mature (Walker, 1980).
7. Uncover the plates and let them air dry. Then, harvest the spores with a cyclone spore collector connected to a small vacuum pump operating at 0.35 bar.

Exercise 5

Method for multiple harvesting of spores

This method has not been examined with Ae5 but it was tested and found successful for other fungi i.e., *Fusarium culmorum, Botrytis* sp., and *Cephalosporium* sp. (Shabana and Charudattan, 2001, submitted). This method can be tested for Ae5 as follows:

1. Grow Ae5 on MRB on a reciprocating shaker (120 strokes/min) at 25 ± 3°C under diurnal light or in continuous darkness for seven to ten days.
2. Under septic conditions, liquefy the contents of each flask in a blender to form a very smooth fungal suspension.
3. Pour 30 ml of this suspension into plastic trays (35 x 30 cm^2) lined with aluminum foil and containing 0.5 cm thick layers (about 500 ml) of V-8 agar medium, supplemented with streptomycin sulphate (0.3 g/ l). Then, cover the trays with plastic wrap and place under two 40 W cool-white fluorescent lamps suspended 45 cm above the trays for a 12-hour light/dark cycleat 27°Cforthreedays.
4. Collect spores on a hood under sterile conditions by adding 50 ml sterile distilled water to each tray and thoroughly rubbing the agar surface with a sterile rubber knife to dislodge the conidia. Place the spore yield in a sterile screw capped bottle and store at 2-4 C if needed.
5. Cover the trays again with plastic wrap and place them under diurnal light for four to five days and reharvest the spores asdescribed.

Phytotoxin production

An important diagnostic characteristic of the Ae5 fungus is the production of crimson red pigmentation in culture under certain conditions **(Figures 13 and 18).** Studies by Charudattan and Rao (1982) have shown the pigmentation to be caused by two compounds (bostrycin and 4- deoxybostrycin) that are phytotoxic to water hyacinth. To determine the optimum cultural conditions for the production of these phytotoxins, six nutritional and environmental factors (culture media, dextrose level, temperature, light regime, aeration and pH) were tested. The production of pigments as well as nonchromatic, UV-absorbing metabolites was determined. The maximum production of pigments was obtained when cultures were grown on PDA containing 20% dextrose, with an initial pH of 4.5 at 25-30°C under continuous darkness or in diurnal light and without wrapping the culture plates (Shabana *et al.,* 2001a). The maximum yields of the nonchromatic UV-absorbing compounds were obtained when cultures were grown on PDA containing 20-50% dextrose, with the initial pH ranging from 3.8 to 6.2 at a temperature range of 20-30°C under continuous darkness on unwrapped plates or on plates sealed with one layer of Parafilm.

The reduction in aeration, presumably proportional to the number of layers of Parafilm wrappings, led to lower levels of the red pigments and the non-pigmented UV-absorbing compounds, reduced mycelial growth and a suppression of sporulation (Shabana, 1992). There was a strong inverse relationship between linear growth and pigmentation as a function of light regime.

Different fractions of culture filtrates of Ae5 were tested for their bioherbicidal activities against water hyacinth in a bioassay **(Figure 19).** Results of the phytotoxin bioassay suggest that a high concentration (10%) of the partially purified culture filtrate was required for symptom expression on water hyacinth. Butanol fractions at 10% (w/v) concentration were the most effective in deteriorating the water hyacinth leaf segments, followed by the aqueous fractions (10% w/v) **(Figure 19).** The area of necrosis increased with time: the damaged leaf area was significantly larger 60 hours after applying the partially purified culture filtrate fractions than it was 30 hours after application (Shabana *et al.,* 2001a).

Physiological and ultrastructural host responses

This work provided interesting details of the host-pathogen relationship between Ae5 and water hyacinth. It also provided good background information to help understand this pathosystem. Infection of water hyacinth with Ae5 led to a significant decrease in pigments, carbohydrates and relative water content and a significant increase in total phenols of water hyacinth leaves. Penetration of water hyacinth leaves by the fungus occurred through the stomata **(Figure 20),** and the invading hyphae were located in the intercellular spaces of leaf tissues. Cytological changes noted in infected cells included changes in chloroplast, nucleus and mitochondria. Invagination of the plasma membrane, particularly at the plasmodesmata, was also noticed in infected cells. The association between the infection process, the physiological disorder and the ultrastructure of infected leaves have been discussed by Shabana *et al.* (1997a).

Formulation and application

A major obstacle to the use of Ae5 as a foliar pathogen for water hyacinth is the need for at least ten hours of dew to enable the fungal propagules to germinate and infect and, to an extent, to colonize the weed (Shabana *et al.,* 1995a). Longer exposure to dew (e.g., 26 or 28 hours) might ensure disease development, but such uninterrupted, extended exposure to dew is not likely to occur under field conditions (Shabana *et al.,* 1995a). For this reason, several approaches have been explored to overcoming the lack of dew in the field by formulating the inoculum in hydrophilic polymers **(Figure 21)** (Shabana *et al.,* 1997b), in invert emulsions (Shabana, 1997a) or in vegetable oil suspension emulsions (Shabana, 1997b). A variety of structurally different invert or oil emulsions have been tested under field conditions to obtain a good, reliable, and cost-effective formula. Formulation also can be used to increase the efficacy of the biological control agents. In this regard, formulating Ae5 along with its phytotoxic fractions in oil emulsions produced 100% weed control seven weeks after application in miniplot trials under field conditions (Shabana, unpublished). The oil prevents dehydration of the fungal inoculum and keeps it moistened for a long time, facilitating the germination of its propagules, while the phytotoxic fractions serve as disease-promoting factors.

Based on recent research by Shabana (unpublished), the oil suspension emulsion of Ae5 that contained 30% cottonseed oil as a final concentration in the end product was found to be the most effective formulation against water hyacinth **(Figures 22 and 24)** out of nine different oil emulsions tested in the absence of dew in greenhouse trials. Work should be continued to test the Ae5 cottonseed formulation at lower ratios of oil, namely 5%, 10%, 15%, 20, 25% and 30%, to determine the most effective formulation that contains the least amount of oil and thus is most cost-effective.

Exercise 6

Preparation of Ae5-alginate formulation

1. Grow Ae5 on PDB at 28°C in the dark for 15 days.
2. Harvest the mycelial mat and rinse it with sterile distilled water.
3. Blend the mycelial mat with an equal amount (w/v) of its culture filtrate for 10 seconds in a blender.
4. Stir the mycelial-filtrate suspension with 1.33% (w/v) sodium alginate in distilled water at 1:4 (v/v). Dissolve sodium alginate in distilled water by stirring for 30 minutes in a 60°C water bath.
5. Drip this preparation into 0.25 m $CaCl_2$ (22.75 g/l distilled water) to form gel beads.
6. Harvest the beads over sieves, rinse with distilled water and spread on carton sheets to be air-dried with the help of electric fans.
7. You may grind the dried pellets into a powder (Figure 25).

Exercise 7

Preparation of Ae5-invert emulsion formulation

(1) Oil phase: consists of: 15% (w/v) paraffin wax, 35% (v/v) paraffin oil, 35% (v/v) soybean oil, 15% (w/v) soybean lecithin (as emulsifying agent)

1. Melt paraffin wax by warming to 50º C in a water bath.
2. Mix with the paraffin oil (oil fraction 1).
3. Add the lecithin to the soybean oil and homogenate in a blender (oil fraction 2).
4. Mix the two oil fractions together with a magnetic stirrer.

(2) Aqueous phase: consists of 1% (w/v) sodium alginate, 0.5% corn syrup, 0.02% Tween 80

Dissolve sodium alginate in distilled water by stirring for 30 min in 60° C water bath. Then, add the corn syrup and Tween and mix together.

(3) Invert emulsion

1. Add the aqueous phase to the oil phase (1:1) and homogenate in a mixer.
2. Add lime [Ca(OH)] at 0.5% (w/v) and the blended Ae5 mycelia to the final invert to give a final concentration of the fungal propagules of 10% (wet w/v). Mix well to ensure homogeneity.

Exercise 8

Preparation of Ae5-cottonseed oil emulsion formulation (Shabana, 2005)

(1) Oil phase

Make 100 ml by blending 75 ml cottonseed oil and 25 ml soybean lecithin (as emulsifying agent).

(2) Aqueous phase

Make 1 500 ml by mixing: 15 g sodium alginate 7.5 ml corn syrup 3 ul Tween 80 160 g of fresh Ae5 mycelia.

Dissolve sodium alginate in distilled water by stirring for 30 minutes in a 60°C water bath. Then, add the corn syrup, Tween and mycelial mat and mix in a blender.

(3) Emulsion

1. Add the aqueous phase (1 500 ml) to the oil phase (100 ml) at 15:1 v/v and homogenate in a mixer.
2. Add 7.5 g lime [Ca(OH)) [at 0.5% (w/v)] and mix well to ensure homogeneity.

Ae5 mycelia (the active ingredient) will be 10% (wet w/v) in the final emulsion while the oil concentration in the final emulsion will be 5% (v/v) (Shabana, 2005).

Exercise 9

Application of oil emulsions on water hyacinth plants

1. Prepare water hyacinth plants for inoculation the day before (as described in Exercise 2) to help the plants settle down and adapt.
2. Spray water hyacinth plants with the Ae5/oil suspension emulsion (as described in Exercise 8) using a hand-held low-pressure sprayer until runoff occurs. Spray control plants with a fungus-free oil emulsion to check its phytotoxic effect in the absence of the fungus.
3. Rate plants for DI and DS at one and eight weeks post treatment. The former (DI) measures the presence of disease (percentage of infected leaves on the plant) and the latter (DS) measures the percent severity of disease damage.
4. Record the dry weight of plants at the end of the experiment and use a proper statistical analysis system to analyze the data.

Gene Manilpulation

Preliminary studies have been initiated to artificially increase the virulence of Ae5 against water hyacinth through genetic manipulation by introducing gene(s) encoded for phytotoxin production or cutinase/cellulase/pectinase enzymes. As a first step, methods have been developed to produce and regenerate protoplasts of the fungus (Shabana and Charudattan, 1997).

Conclusions

Alternaria eichhorniae 5 is a safe and effective bioherbicide for water hyacinth. High levels of bioherbicidal efficacy and weed stress were seen when an alginate formulation of mycelium and culture filtrate of Ae5 was used along with a polyacrylamide. Nonetheless, this formulation was deemed insufficient to overcome the weed's growth rate conditions without the benefit of prolonged dew periods. Formulating the fungal inoculum in an invert emulsion helped to overcome this obstacle by causing high disease severity levels on water hyacinth plants even without exposure to dew. Practically speaking, the formulation of the fungal inoculum in invert emulsions has a number of disadvantages: the large amount of oil required adds greatly to the cost of the product; the material is very viscous and requires special equipment for application; and non-target contamination by the petroleum oil and other components of the formulation is likely. Therefore, the potential of emulsions with low oil content and emulsions made from vegetable oils was investigated. The rapeseed (Shabana, 1997b) and cottonseed oil (Shabana, unpublished) suspension emulsions are promising under dew-free conditions. Evaluations of these products in large-scale field trials are necessary to confirm the results achieved in miniplot trials.

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Figure 1. Chlorotic flecks and necrotic lesions on a water hyacinth leaf five days after inoculation with a conidial suspension of Ae5.

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Figure 2. Chlorotic lesions with varying degrees of necrosis and coalescence of lesions on a water hyacinth leaf eight days after inoculation with mycelial suspension of Ae5 containing a humectant.

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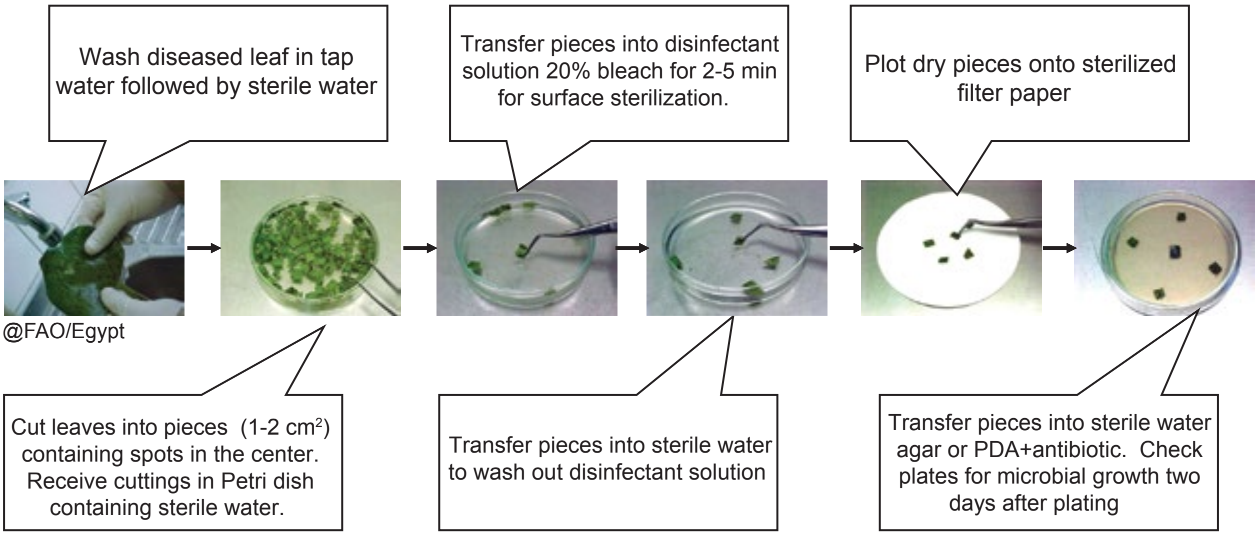
Figure 3. Water hyacinth leaf showing leaf blight 12 days after inoculation with mycelial suspension of Ae5 containing a humectant.

Figuer 4. Steps for isolation of causal organisms from diseased water hyacinth leaves



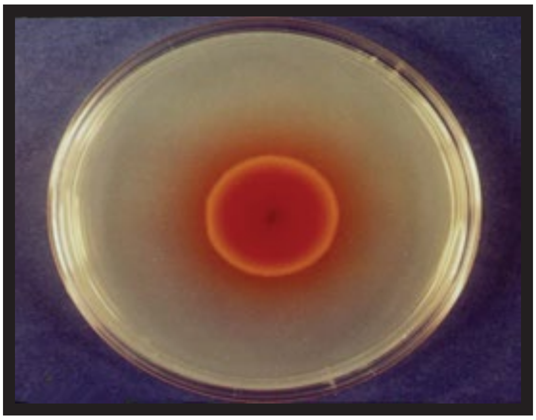


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Figure 5. A pure culture of *Alternaria eichhorniae* 5 on potato dextrose agar. Note the crimson red pigment secreted by the fungus in the medium.

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Figure 6. Notice the coloration of the culture filtrate of Ae5 (left) and *A. alternata* (right) grown on potato dextrose broth.

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Figure 7. Preliminary test for determination of the pathogenicity of a fungal strain isolated from diseased water hyacinth leaves. The top edge of the polyethylene bag is stapled (left) to maintain high relative humidity.

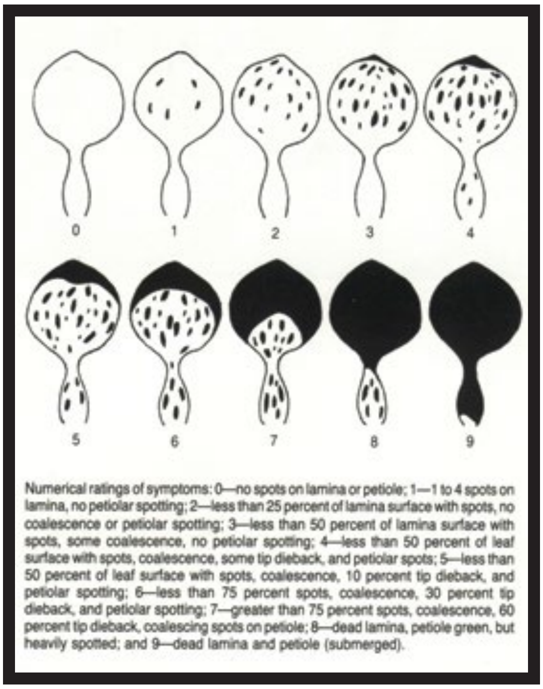

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Figure 8. Pictorial disease scale on water hyacinth leaves (Freeman and Charudattan, 1984).

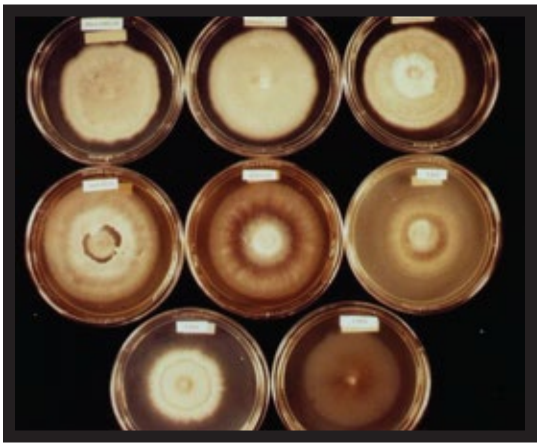

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Figure 9. Effect of medium on the growth of Ae5. top row: Difeo potato dextrose agar; fresh potato dextrose agar; fresh potato dextrose yeast extract agar; middle row : water hyacinth-potato dextrose agar; water hyacinth dextrose agar; lima bean agar; bottom row: Czapek-Dox agar; and corn meal agar. The hydrogen ion concentration of each medium was adjusted to pH 7 ± 0.1 after autoclaving with sterile solutions of 1 N and/or 50% (w/v) NaOH and HCl. The cultures were incubated at 25°C in the dark.

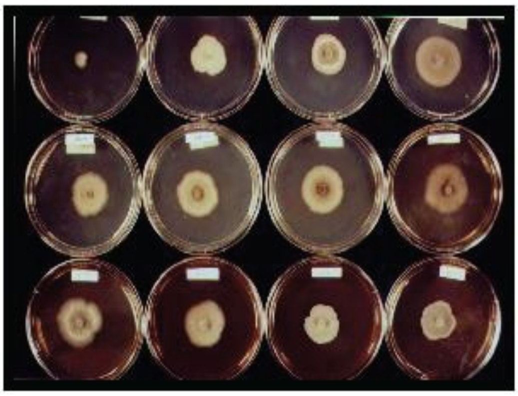

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Figure 10. Effect of pH on the growth of Ae5 on PDA,
top row : pHs of 4, 4.6, 5, and 5.6
middle row: pHs of 6,6.5, 7, and 7.5
bottom row : pHs of 8,8.7,9, and 9.7
The pH levels from 4.0 to 7.5 were adjusted with citric acid-Na HPQ (McIlvaine) buffer solutions and pH levels from 8 to 9.7 were adjusted with Clark and Lubs buffer solutions (Dawson *et al.,* 1969). In each case, the pH was adjusted before auto-claving. The cultures were incubated at 25°C in the dark.

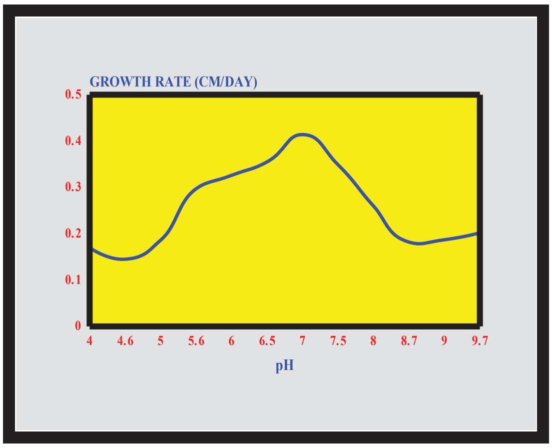

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Figure 11. Effect of pH on the growth rate of Ae5 on PDA. The solid line represents the predicted values of the growth rate (cm/day) and the dots are the observed values (of seven replicates) of the growth rate.

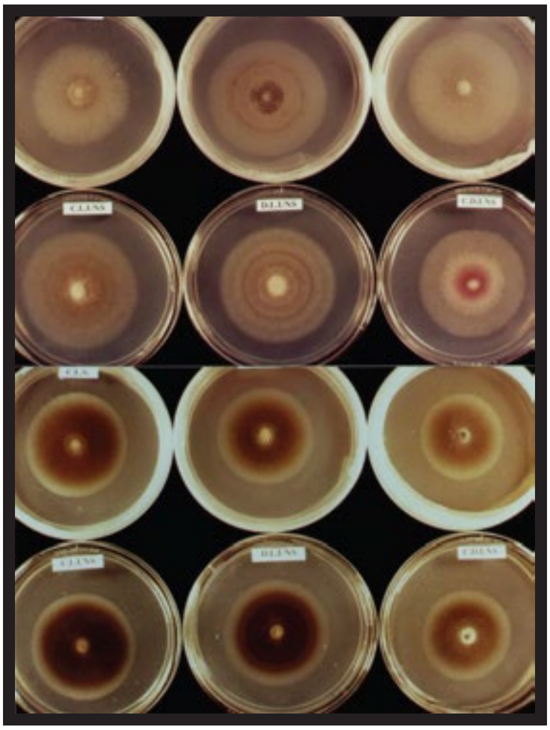

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Figure 12. Effect of incubation temperature, plate-sealing and light regime on the growth of Ae5, on 5% V-8 juice, 2% agar (5/2 agar). First row (top): sealed culture grown at 30°C under continuous light (CL); sealed culture grown at 30°C under diurnal light (DL); sealed culture grown at 30°C under continuous darkness (CD); Second row: unsealed culture grown at 30°C under CL; unsealed culture grown at 30°C under DL; unsealed culture grown at 30°C under CD; third row: sealed culture grown at 22°C under CL; sealed culture grown at 22°C DL; sealed culture grown at 22°C under CD; fourth row (bottom): unsealed culture grown at 22°C under CL; unsealed culture grown at 22°C under DL; unsealed culture grown at 22°C under CD. The photograph was taken five days after inoculation.

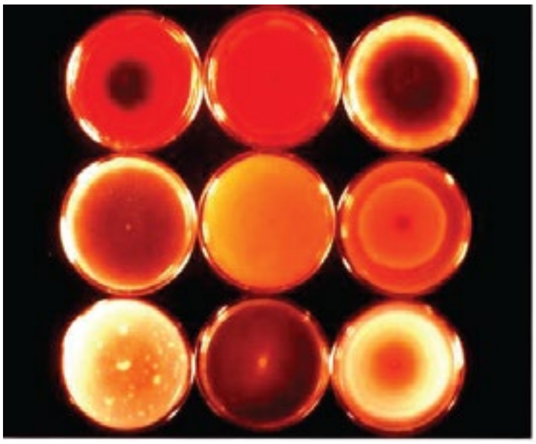

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Figure 13. Effect of nine media on pigmentation and growth of Ae5. Left to right, starting from the upper row: potato dextrose agar difco (PDA); potato-water hyacinth dextrose agar (4:1 v/v, respectively) (PDAWH 20%); potato water hyacinth dextrose agar (3:2 v/v, respectively) (PDAWH 60%); potato water hyacinth dextrose agar (1:4 v/v, respectively) (PDAWH80%); V-8 juice agar (V-8A); modified Richard's agar (MRA); oat meal agar (OMA); corn meal agar (CMA); and Czapek- Dox agar (CDA). The cultures were kept unsealed and incubated at 28°C in the dark. The photograph was taken ten days after inoculation.

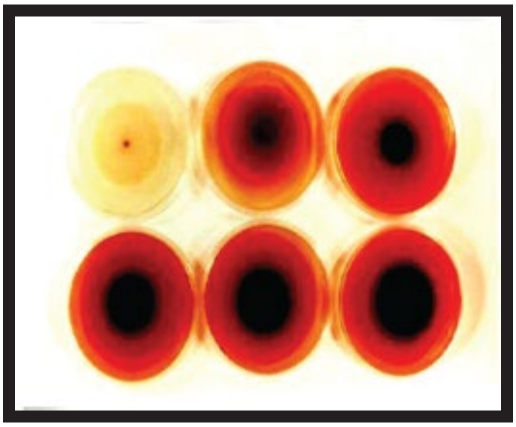

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Figure 14. Effect of dextrose level in the medium (potato agar) on pigmentation and growth of Ae5. Left to right, starting from the upper row: 0, 10, 20, 30, 40 and 50 g/l of dextrose. The cultures were kept unsealed and incubated at 28°C in the dark. The photograph was taken seven days after inoculation.

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Figure 15. Effect of incubation temperature on pigmentation and growth of Ae5 on PDA. Left to right, starting from the upper row: 15°C, 20°C, 25°C, 26°C, 28°C, 30 and 35°C. The cultures were kept unsealed and incubated in the dark. The photograph was taken ten days after inoculation.

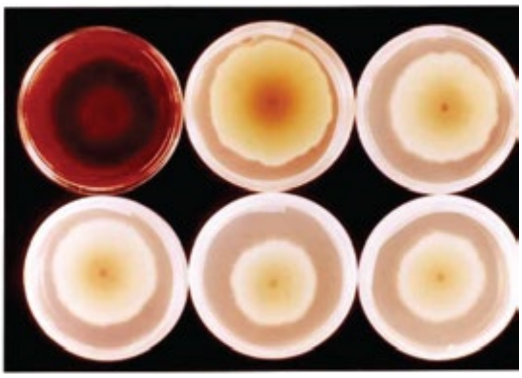

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Figure16. Effectofaerationon pigmentation and growth of Ae5 on PDA. Plates were left unsealed or sealed with Parafilm. Left to right, starting from the upper row: unsealed, one layer of Parafilm, two layers, four layers, eight layers and 12 layers. The cultures were incubated at 28°C in the dark. The photograph was taken ten days after inoculation.

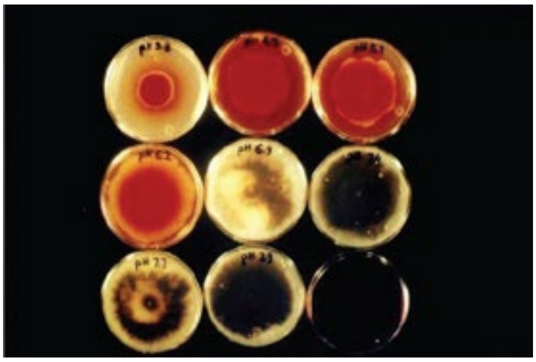

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Figure 17. Effect of pH on pigmentation and growth of Ae5 on PDA. The pH of the medium was adjusted with 1N and/or 50% (w/v) NaOH and 1N HCl before autoclaving and was measured after autoclaving. The cultures were kept unsealed and incubated at 28°C in the dark. The photograph was taken eight days after inoculation.

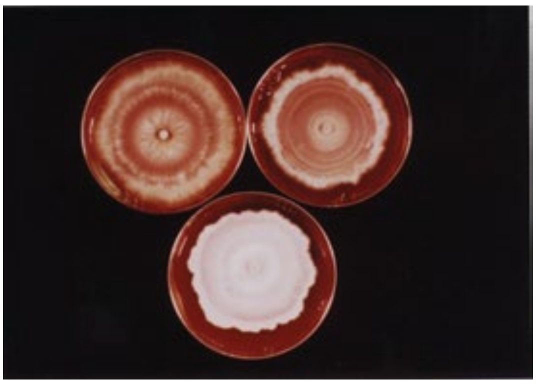

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Figure 18. Effect of light regime during culturing on pigmentation and growth of Ae5 on PDA. Clockwise from upper left: continuous light; diurnal light; and continuous darkness. The cultures were unsealed and incubated at 28°C. The photograph was taken ten days after inoculation.

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Figure 19. A bioassay to test the bioherbicidal activities of culture filtrate of Ae5 on leaf segments of water hyacinth. Left to right: potato dextrose broth (control), aqueous fraction 10% (upper row) butanol fraction I (10%), and butanol fraction II (10%) (lower row).

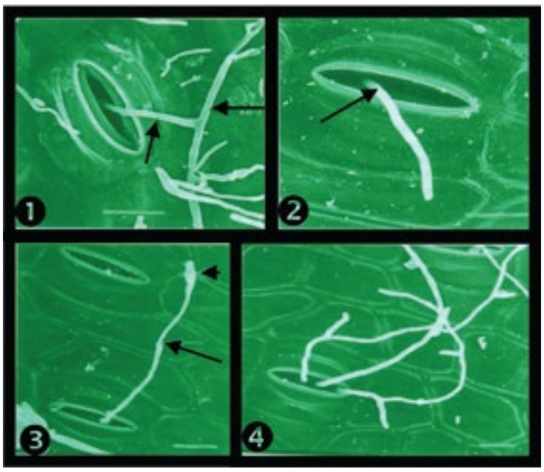

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Figure 20. Scanning electron micrographs of a water hyacinth leaf treated with alginate formulation of mycelium plus culture filtrate of Ae5:

(1) mycelial branch (arrow) arising from mycelium (MY) entering the leaf tissue through a stoma;

(2) Growing conidiophore emerging from a stoma. Note the septum (arrow);

(3) Mature conidiophore emerging from a stoma. Note the septum (arrow) and the formation of a spore (arrowhead);

(4) Three branched conidiophores emerging from one stoma.

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Figure 21. Chlorotic lesions and leaf blight on water hyacinth plants inoculated with alginate formulation of Ae5, two weeks after treatment under field conditions.

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Figure 22. Severe leaf and petiole blight on water hyacinth plants inoculated with vegetable oil suspension emulsion of Ae5, three weeks after treatment under field conditions.

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Figure 23. Deterioration of water hyacinth plants inoculated with vegetable oil suspension emulsion of Ae5, five weeks after treatment under field conditions.

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Figure 24. A 100% Control of water hyacinth in a filed plot, seven weeks after inoculation with vegetable oil suspension emulsion formulation of Ae5 under field conditions.

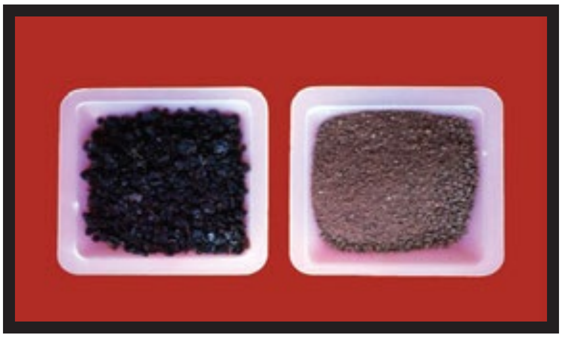

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Figure 25. Granules (left) and powder (right) of Ae5 formulated in sodium alginate.

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