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THE USE OF OIL EMULSIONS FOR IMPROVING THE EFFICACY OF *Alternaria eichhorniae* MYCOHERBICIDE ON WATER HYACINTH

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ABSTRACT

The fungus *Alternaria eichhorniae* isolate #5 (Ae5) is being developed as a mycoherbicide agent for controlling water hyacinth (*Eichhornia crassipes*) in Egypt. Inoculum concentrations above 10% (mycelial wet weight) were equally effective in the management of water hyacinth. There was an inverse relationship between the fungal culture age (up to 9 week old) and inoculum infectivity; the younger mycelial inoculum was more virulent on the weed plants than older ones. Applications of Ae5 in an aqueous carrier and/or in nine different invert/oil emulsions were evaluated for the biocontrol of water hyacinth without a post-treatment dew period under natural (outdoor) conditions. The invert/oil emulsions were developed to provide moisture and to retard evaporation from the fungal inoculum applied so that the fungal propagules could germinate and infect the target weed under relatively dry conditions. All of the invert and/or oil emulsion formulations induced higher levels of disease on water hyacinth plants when compared to the aqueous suspension formulation under dew-free conditions. Reducing the oil content in the Ae5 emulsion formulation from 30 to 5% had no significant effect on the formulation's infectivity against water hyacinth. In the absence of dew, Ae5 formulated in cottonseed oil emulsion caused 100% control of water hyacinth in the outdoor field plots 7 to 13 weeks after application. Thus, the results confirm the utility and feasibility of oil emulsions as formulating materials for bioherbicides under the lack of dew conditions.

INTRODUCTION

Water hyacinth [*Eichhornia crassipes* (Mart.) Solms], the world's worst aquatic weed, causes annual losses to hydro-electricity generation, irrigation schemes, fisheries, riparian communities and water transport in excess of US$100 million. Thick mats of water hyacinth which can 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 infested area with water hyacinth is estimated to be 638 km^2 covering most of the lakes, drainage and irrigation canals (Fayad *et al.*, 2001). The total amount of water loss by evapotranspiration from this infested area was estimated to be 3.5 billion m^3 per year. This amount is sufficient to irrigate a further 432 km^2 every year (Fayad *et al.*, 2001). This issue became more significant in view of the fact that the Egyptian demand for irrigation water is increasing as a consequence of ever-increasing the new 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, and human health and water supplies. It has also major impacts on related economic activities and community livelihoods.

Resource-poor communities that rely on lakes and rivers for their survival are severely affected by this noxious weed through reduced access to water, reduced water quality, increased health problems from bilharzias, malaria, cholera and snakes, and a reduction in household income through a decline in fishing effort.

Since 1990, the Egyptian authorities have banned the use of chemical herbicides in all water bodies in Egypt due to environmental and health concerns. At present, the weed is controlled through mechanical or manual removal which running costs are more than US$ 7 million every year (an estimate cost of 1993, Labrada, 1993). Physical removal does not provide any significant reduction of weed stands and water hyacinth continues to be a serious problem. Therefore, there is a need for a control technology of the weed which combines the environmental safety of biological control with the speed of chemical control. The development of biological herbicides that use naturally occurring fungal diseases affect only water hyacinth (mycoherbicides) is a very promising control option. Recent studies in Egypt have demonstrated that suitable fungi with a high degree of specificity to water hyacinth and good levels of pathogenicity already exist in Egypt and these may work with existing insect agents in the biological control of water hyacinth.

Alternaria eichhorniae Nag Raj & Ponnappa, a fungal pathogen of water hyacinth, has been reported on water hyacinth in many countries in the world namely, Egypt, Sudan, Kenya, Zimbabwe, Ghana, Uganda, Niger, Tanzania, South Africa, India, Indonesia, and Thailand. This fungus has shown to be fairly host-specific to water hyacinth (Nag Raj and Ponnappa, 1970; Rakvidhyasastra *et al.*, 1978; and Shabana *et al.*, 1995a) 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.*, 1995a, b; 1997a, b; 2000; 2001). Based on the work of Shabana *et al.* (1995a), a major obstacle to the use of Ae5, as a foliar pathogen for water hyacinth, is the need for at least 10 h of dew to enable the fungal propagules to germinate and infect, and to an extent, to colonize the weed. Longer exposure to dew (e.g., of 26 or 28 h) might assure disease development, but such uninterrupted, extended exposure to dew periods is not likely to occur under field conditions. For this reason, several thoughts have been explored and examined to overcome the lack of dew under the field conditions by formulating the fungal inoculum in hydrophilic polymers (Shabana *et al.* 1997b), by formulating the inoculum in invert emulsions (Shabana 1997a), or in vegetable oil suspension emulsions (Shabana 1997b). However, formulating Ae5 in the invert emulsions and/or in vegetable oil suspension emulsions averted dew dependence in greenhouse trials, a variety of structurally different invert emulsions needed to be evaluated under field conditions to obtain a good, reliable, and cost effective formula for the biocontrol of water hyacinth on the large scale. Hence, practical and economical utilization of this fungus as bioherbicide under field conditions was the major objective of the present investigation. Therefore, the specific objectives of this research were: 1) screen a wide range of structurally different invert and/or oil emulsion formulations to evaluate their

biocontrol efficacy against water hyacinth in the field; 2) determine the inoculum threshold of the bioherbicide; 3) study the effect of culture age of Ae5 on the inoculum infectivity; and 4) attempting to reduce the oil content in the Ae5 emulsion bioherbicide to the limit that does not affect its infectivity against water hyacinth.

Technical results of this research are expected to help us: 1) diversify our weed control options, 2) reduce dependency on expensive practices for water hyacinth control, and 3) offer an environmentally beneficial weed management tool. The success of this program could have worldwide significance because of the worldwide importance of water hyacinth.

MATERIALS AND METHODS

1. Evaluation of different invert emulsion and/or vegetable oil suspension emulsion formulations of Ae5 for their biocontrol efficacy against water hyacinth in the absence of dew

Nine different oil phases were prepared: (1) a blend of 10% (w/v) paraffin wax, 20% (w/v) soybean lecithin, and 70% (v/v) sunflower oil; (2) 15% paraffin wax, 15% soybean lecithin, 35% sunflower oil, and 35% cotton seed oil; (3) 15% paraffin wax, 35% soybean lecithin, 25% sunflower oil, 25% cotton seed oil; (4) 20% paraffin wax, 20% soybean lecithin, and 60% sunflower oil; (5) 25% soybean lecithin and 75% cotton seed oil; (6) 15% soybean lecithin and 85% sunflower oil; (7) 15% soybean lecithin and 85% cotton seed oil; (8) 15% soybean lecithin and 85% paraffin oil; and (9) 10% (w/v) lanolin, 15% soybean lecithin, 5% (w/v) bees wax, and 70% sunflower oil. In this study lecithin was used as the emulsifying agent, and paraffin oil and wax and vegetable oils were used at different ratios to retard evaporation further and help retain droplet size (Shabana 1997a, b). The paraffin wax was melted by warming to 50C and then mixed with the mineral oil. Lecithin was added to the vegetable oil and homogenized in a blender. The two oil fractions were mixed with a magnetic stirrer. The aqueous phase contained 1% (w/v) sodium alginate, 0.5% corn syrup, and 0.02% Tween 80. In each case, the aqueous phase was added to the oil phase (1 : 1), except in treatment # 5 where the ratio of oil phase/aqueous phase was 2/3 (v/v), and mixed in a mixer. Lime [Ca $(OH)_2$] at 0.5% (w/v) and blended Ae5 mycelia were added to the final invert to give a final concentration of the fungal propagules of 10% (wet w/v) and mixed well on a stirrer to insure homogeneity.

Medium size, 5- to 7-leaf water hyacinth plants were collected from the Nile River, trimmed of any necrotic or senescent tissues, and maintained for a week in outdoor concrete pools (1 x1.5 m^2) containing river water. Plants were transferred a day before inoculation to large plastic vats (60 cm diameter x 22 cm depth) containing 15 l river water (500 g plant fresh weight per vat). The fungus formulations in invert emulsions and /or in vegetable oil suspension emulsions were applied to water hyacinth plants using air assist sprayer or back-held sprayer, respectively, and sprayed until incipient runoff occurred. Control plants were sprayed with the water-based suspension

(10% w/v mycelial suspension in the aqueous phase). Three replicates were used for each treatment and arranged in a completely randomized design. Three plants were also sprayed with each invert emulsion, vegetable oil suspension emulsion, and/or aqueous carrier only (without fungus), to check for phytotoxic effects in the absence of the fungus. Plants were rated 1 and 8 weeks post treatment for disease incidence (DI) and disease severity (DS), the former as the presence of disease (percentage of infected leaves on the plant) and the latter as the percent severity of disease damage. Number of leaves and fresh weight of water hyacinth plants in each replicate were recorded at the zero time as well as at the end of the experiment (8 weeks after treatment). Number of dead leaves, accumulated water loss, and the dry weight of plants were recorded at the end of experiment.

2. Determination of optimum inoculum concentration

The most effective formulation obtained as a result of the previous experiment (the fungus formulated in oil phase # 5; treatment # 5) was tested further in the greenhouse using different inoculum densities namely, 0, 1, 5, 10, 15, 20, and 25% (wet w/v) (final concentration of the fungal propagules in the formulation) in order to determine the inoculum threshold of the mycoherbicide (the inoculum concentration necessary for effective control). Water hyacinth plants were prepared as mentioned above. Five replicates (plastic vats) were used for each inoculum level and arranged in a completely randomized design. Back-held sprayer was used for applying the fungal formulation onto water hyacinth plants. DI and DS were determined 10 days after treatment.

3. Evaluation of the cottonseed oil-based formulation using the optimum inoculum density on water hyacinth in a vat study and outdoor field plots in two seasons

A. 1998 trial in outdoor field plots

The most effective formulation obtained as a result of first experiment mentioned above (cottonseed oil-based formulation at 30% oil content) at the optimum inoculum level obtained as a result of the second experiment (10% inoculum concentration) was tested in outdoor 1.5 x 1 m^2 concrete pools containing approximately 15 kg of healthy, medium size water hyacinth plants. Back-held sprayer was used for applying the fungal formulation onto water hyacinth plants until incipient runoff occurred (on November 15, 1998). Control plants were sprayed with the fungal-free emulsion. Five replicates were used for each treatment and arranged in a completely randomized design. DS was determined 3, 5 and 7 weeks after treatment.

B. 2002 trial in outdoor large vats

Identical formulation to the one used in 1998 study was examined in this experiment. However, one problem was experienced prior to the preparation for this trial, which was the loss of virulence of Ae5. This was probably, due to increased numbers of subculturing during the long period of preservation (about 3 years). Therefore, subsequent passages of Ae5 through water hyacinth plants were conducted to recover its virulence before the initiation of this trial.

Medium size, healthy looking, 5- to 7-leaf water hyacinth plants were collected from the Nile River and arranged in large plastic vats (60 cm diameter x 22 cm depth) containing 15 l river water (500 g plant fresh weight per vat). Water hyacinth plants were inoculated with Ae5 formulated in cottonseed oil emulsion using a back-held sprayer and spraying until incipient runoff occurred (on 23 January 2002). Control plants were sprayed with fungus-free cottonseed oil emulsion, and/or aqueous carrier only (without fungus), to check for phytotoxic effects in the absence of the fungus. Four replicates were used for each treatment and arranged in a completely randomized design. Plants were rated 3 weeks post treatment and then every 2 weeks up to the 11th week for DI and DS.

C. 2002 trial in outdoor field plots

The same formulation used in the above vat study was evaluated here in outdoor field plots and applied onto water hyacinth plants on January 23, 2002. Three replicates were used for each treatment and arranged in a completely randomized design. DS was determined 3 weeks after treatment and then every 2 weeks up to 13 weeks.

4. Effect of culture age of Ae5 on the inoculum infectivity

Inocula were prepared from Ae5 broth cultures grown on Difco potato dextrose broth (DPDB, DIFCO Laboratories, Detroit, MI, USA) for 4, 9, and 16 weeks at 28 C in the dark. Two types of inocula were prepared: the first one was mycelial suspension in sterile water (20% w/v) and the second type was mycelial suspension in its culture filtrate (20% w/v).

Medium size, 5- to 7-leaf water hyacinth plants were collected from the Nile River and maintained for a week in outdoor concrete pools (1 x1.5 m^2) containing river water. Plants were transferred a day before inoculation to plastic pots (one plant per pot of 9 cm diameter and 12 cm depth) containing 400 ml of tap water. Plants were sprayed with each inoculum using a hand-held low-pressure sprayer and then covered with clear polyethylene bags for 48 h to maintain a high relative humidity. Plants sprayed with sterile water without fungus served as a control. There were five replicates per treatment, which were arranged in a completely randomized design. Five days after inoculation, the plants were rated for DI and DS. DS was determined for each leaf with the help of a pictorial disease scale of 0 to 9, where 0 = healthy and 9 = 90% diseased (Freeman and Charudattan, 1984). Values for individual leaves were summed and averaged to derive DS as percentages for a whole plant.

5. Effect of the oil content in the Ae5 emulsion formulation on its infectivity against water hyacinth

Cottonseed oil-based emulsion formulation was tested using different oil concentrations (0, 5, 10, 15, 20, 25, and 30% cottonseed oil content in the final formulation) in order to determine the least oil concentration in the formulation that can produce the highest level of DS on water hyacinth. Water hyacinth plants were prepared in pots as described. Three replicates were used for each treatment and arranged in a completely randomized design. Hand-held low-pressure sprayer was used for application of the

formulations onto water hyacinth plants. Three types of control plants were set; "control I" plants were sprayed with the fungus-free emulsion, "control II" plants were sprayed with the aqueous carrier containing the fungus (0% oil content) but not exposed to the dew after inoculation, and "control III" plants were sprayed with the aqueous carrier containing the fungus (0% oil content) but exposed to the dew after inoculation. Dew was provided, post-treatment, by covering plants with clear polyethylene bags atomized with water from internal walls for 48 h and then uncovered. Plants treated with Ae5 formulated in oil emulsion (aqueous carrier + oil phase) were kept uncovered after treatment (did not exposed to dew) on a bench in the laboratory (25 ± 3 C, about 48% RH and approximately 270 $\mu E/m^2.s$ light at midday). DI and DS were determined 10 days after treatment.

Statistical analyses

The data were analyzed using SAS software package (SAS Institute, 1996). All multiple comparisons were first subjected to ANOVA. Significant differences among treatment means were determined with Tukey's studentized range test, Duncan's new multiple range test, or LS means test as appropriate.

RESULTS

1. **Evaluation of different invert emulsion and/or vegetable oil suspension emulsion formulations of Ae5 for their biocontrol efficacy against water hyacinth in the absence of dew:**

Fungal inoculum applied in the formulation #5 (contained 30% cottonseed oil) proved to be the most effective formulation in managing water hyacinth in the vat study (Table 1). It caused the least fresh and dry weight of water hyacinth plants (97.66 and 10.33 g, respectively), the least amount of water loss (12.43 L)and the highest DI and DS on weed plants (100% and 95% for DI and DS, respectively) 8 weeks after inoculation in the absence of dew. With regard to DS and DI, the greatest difference between application in water and emulsions was found under severe conditions (when no dew was provided). In the absence of dew, the invert emulsion formulations and the vegetable oil suspension emulsion formulations were significantly better than the aqueous formulation in promoting DS and DI ($P = 0.05$) (Table 1). There was no constant relationship between DI and DS since disease can occur on all leaves of the plant (DI = 100%) although its severity may be low (low DS). However, there was an inverse relationship between the DS and the accumulation of water loss (Table 1). The amount of accumulated water loss recorded in the control (treatment # 14) was approximately 6 times more than the amount lost in the most effective treatment (cottonseed oil formulation, treatment # 5) (74.2 versus 12.4 liter, respectively) (Table 1).

Table 1. Evaluation of *Alternaria eichhorniae* 5 (Ae5) bioherbicidial formulations on water hyacinth plants in a vat study.

Treatment[y]	No. of leaves at Zero time	No. of leaves at end	No. of dead leaves at end	Fresh weight at end (g)	Dry weight (g)	Accumulated water loss (L)	DI % after 1 week	DS % after 1 week	DI % after 8 weeks	DS % after 8 weeks
1	64.33 a[z]	48.00 de	37.33 a	129.33 e	14.66 e	18.33 de	80.00 bc	33.33 b	96.66 ab	75.00 c
2	64.00 a	50.33 d	39.33 a	135.66 e	17.33 de	20.60 d	76.67 c	30.00 b	90.00 bc	71.66 c
3	58.66 a	41.66 de	30.00 ab	149.00 de	17.33 de	17.80 de	80.00 bc	30.00 b	86.66 cd	76.66 c
4	63.66 a	50.00 d	31.33 ab	139.33 e	15.00 e	18.03 de	85.00 ab	31.66 b	81.66 d	75.55 c
5	61.66 a	25.00 e	21.33 bc	97.66 e	10.33 e	12.43 e	90.00 a	46.66 a	100.00 a	95.00 a
6	61.33 a	43.33 de	24.00 bc	113.66 e	16.33 de	16.83 de	88.33 ab	35.00 b	83.33 cd	76.66 c
7	64.66 a	34.00 de	21.00 bc	112.66 e	11.53 e	15.16 de	88.33 ab	43.33 a	100.00 a	86.66 b
8	67.33 a	36.00 de	24.00 bc	113.33 e	11.00 e	15.33 de	90.00 a	43.33 a	100.00 a	88.33 b
9	60.33 a	45.00 de	23.66 bc	138.00 e	14.76 e	19.63 d	86.67 ab	30.00 b	80.00 d	71.66 c
10	66.33 a	79.00 c	19.00 cd	216.33 d	22.23 d	31.70 c	56.67 d	16.66 c	70.00 e	65.00 d
11	68.33 a	98.66 bc	10.66 d	2114.33 b	151.16 a	60.33 b	11.66 e	3.00 d	15.00 fg	8.33 f
12	63.66 a	100.33 bc	9.66 d	2042.33 c	138.10 b	62.56 b	11.66 e	3.00 d	15.00 fg	15.00 e
13	67.33 a	114.00 ab	9.33 d	2117.66 b	120.60 c	70.70 a	11.66 e	2.00 d	16.66 f	11.66 ef
14	68.66 a	125.33 a	10.33 d	2390.66 a	155.40 a	74.20 a	3.33 f	1.00 d	8.33 g	6.66 f

[y] **The treatments from 1-9: the inoculum consisted of the oil phase + the aqueous phase + the fungus. The nine different oil phases that were used composed, respectively, of: (1) 10% (w/v) paraffin wax, 20% (w/v) soybean lecithin, and 70% (v/v) sunflower oil; (2) 15% paraffin wax, 15% soybean lecithin, 35% sunflower oil, and 35% cotton seed oil; (3) 15% paraffin wax, 35% soybean lecithin, 25% sunflower oil, 25% cotton seed oil; (4) 20% paraffin wax, 20% soybean lecithin, and 60% sunflower oil; (5) 25% soybean lecithin and 75% cotton seed oil; (6) 15% soybean lecithin and 85% sunflower oil; (7) 15% soybean lecithin and 85% cotton seed oil; (8) 15% soybean lecithin and 85% paraffin oil; and (9) 10% (w/v) lanolin, 15% soybean lecithin, 5% (w/v) bees wax, and 70% sunflower oil. The treatment #10 composed of the aqueous phase only containing the fungus (Check #1). The treatments 11-14 composed of different oil phases only without the fungus (Checks # 2, 3, 4, 5).**

[z] **Means within a column followed by the same letter(s) are not significantly different according to Duncan's Multiple Range test (P = 0.05)**

2. Determination of the optimum inoculum concentration

According to an ANOVA, inoculum concentration had a significant effect ($P = 0.0001$) on efficacy. Two weeks after inoculation, DS increased with uprising the inoculum concentration up to 10% mycelial inoculum and then remained at the same DS level up to 25% (Table 2).

Table (2): Effect of inoculum concentration of *Alternaria eichhorniae* 5 (Ae5) on water hyacinth plants in a vat study 10 days after application

Inoculum concentration(% w/v) [w]	Disease incidence (%) [x]	Disease Severity (%) [y]
0 (Check)	3 b [z]	1 d
1	75 a	50 c
5	79 a	63 b
10	81 a	77 a
15	87 a	76 a
20	83 a	76 a
25	86 a	77 a

3. Evaluation of the cottonseed oil-based formulation using the optimum inoculum density on water hyacinth in a vat study and outdoor field plots in two seasons

A. 1998 trial in outdoor field plots

According to an ANOVA, the treatment had a significant effect ($P = 0.0001$) on DS. The DS on inoculated plants increased significantly with time after inoculation. About 5 weeks after application, there was a cross-contamination of control plants from inoculated plants, which took place most likely by wind (Table 3). Severe leaf and petiole blight appeared on inoculated water hyacinth plants 3 weeks after application (Fig. 1). Deterioration of water hyacinth plants in inoculated plots was clearly manifested 5 weeks post treatment (Fig. 1). A 100% control of water hyacinth has occurred 7 weeks post inoculation under field conditions (Table 3, Fig. 1).

Table (3): Effect of cottonseed oil-based formulation of *Alternaria eichhorniae* 5 (Ae5) sprayed on water hyacinth in outdoor field plots on 15 November 1998

Treatment	Disease Severity %		
	After 3 weeks	After 5 weeks	After 7 weeks
Noninoculated Control	3 b [z]	15 b	30 b
Inoculated	70 a	90 a	100 a

[z] Means within a column followed by the same letter are not significantly different according to Duncan's Multiple Range test ($P = 0.05$).

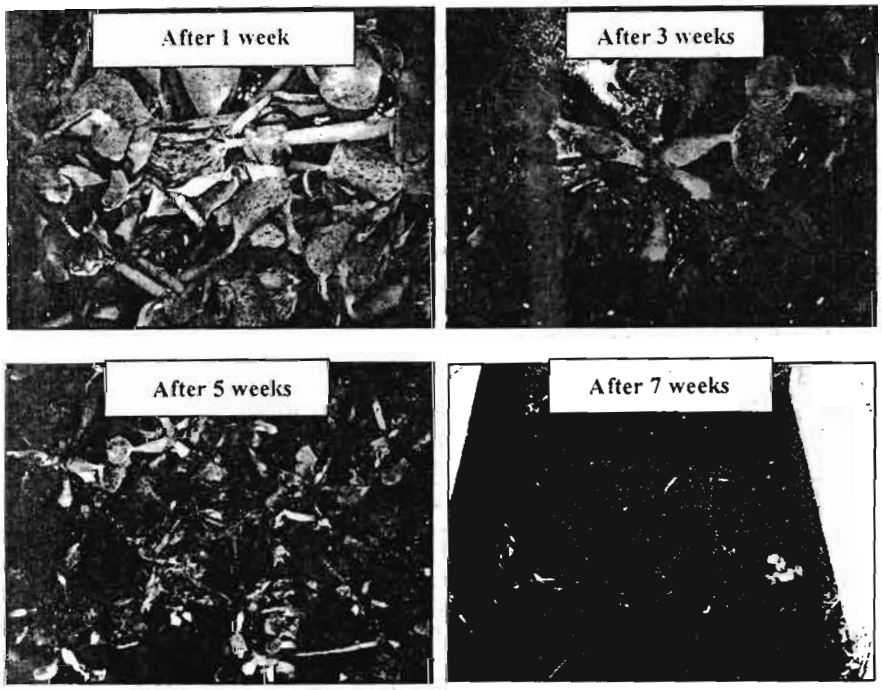


Fig. 1. Performance of Ae5 mycoherbicide (formulated in cottonseed oil emulsion) sprayed on water hyacinth plants on November 15, 1998 in outdoor field plots. A 100% weed kill was obtained 7 weeks after application.

B. 2002 trial in outdoor large vats

According to an ANOVA, the treatment had a significant effect (P = 0.0001) on DI and DS. The DI as well as the DS on inoculated plants increased significantly with time after inoculation until 11 weeks post application. Ae5 formulated in cottonseed oil emulsion caused 100% weed kill 11 weeks after inoculation in the absence of dew (Table 4, Fig. 2). These results confirmed the findings obtained from 1998 season trials.

Table 4. Effect of cottonseed oil-based formulation of *Alternaria eichhorniae* 5 (Ae5) sprayed on water hyacinth plants on 15 November 1998 in a vat test

Treatment	Disease incidence (DI, %) after week(s)						Disease severity (DS, %) after week(s)					
	0	3	5	7	9	11	0	3	5	7	9	11
Check I (no fungus/no oil)	0 j[a]	0 j	0 j	0 j	16.3 hi	26.3 f	0 h	0 h	0 h	0 h	5.8 fg	8.8 f
Check II (oil phase only)	0 j	18.8 gh	18.8 gh	12.5 I	22.5 fg	41.3 e	0 h	6.3 f	5.8 fg	2 gh	5.8 fg	8 f
Ae5 emulsion formulation	0 j	70 d	75 d	82.5 c	92.5 b	100 a	0 h	22.5 e	37.5 d	65 c	81.3 b	100 a

[a] **Values of DI or DS followed by the same letter(s) are not significantly different according to LS means test (P < 0.03 for DI and ≤ 0.05 for DS).**

C. 2002 trial in outdoor field plots

According to an ANOVA, the treatment had a significant effect (P = 0.0001) on DS. The DS on inoculated plants increased significantly with time after inoculation until 11 weeks post application. Severe leaf and petiole blight on inoculated water hyacinth plants appeared 3-5 weeks after application (Fig. 3). Deterioration of water hyacinth plants in inoculated plots was apparent 7 weeks post treatment and a 100% control was obtained 13 weeks post treatment under field conditions (Table 5, Fig. 3).

Table (5): Effect of cottonseed oil-based formulation of *Alternaria eichhorniae* 5 (Ae5) s prayed o n w ater h yacinth in outdoor field plots on 15 November 1998.

Treatment	Disease Severity (%) after week(s)						
	0	3	5	7	9	11	13
Check I(fungus-free aqueous carrier)	0 f[a]	0 f	0 f	0 f	6 f	6.7 ef	6.7 ef
Check II (oil phase only)	0 f	5 f	2.7 f	1.3 f	2.7 f	6 f	10 ef
Ae5 emulsion formulation	0 f	15 e	35 d	65 c	81.7 b	93.3 a	100 a

[a] **Values followed by the same letter(s) are not significantly different according to LS means test (P ≤ 0.04).**

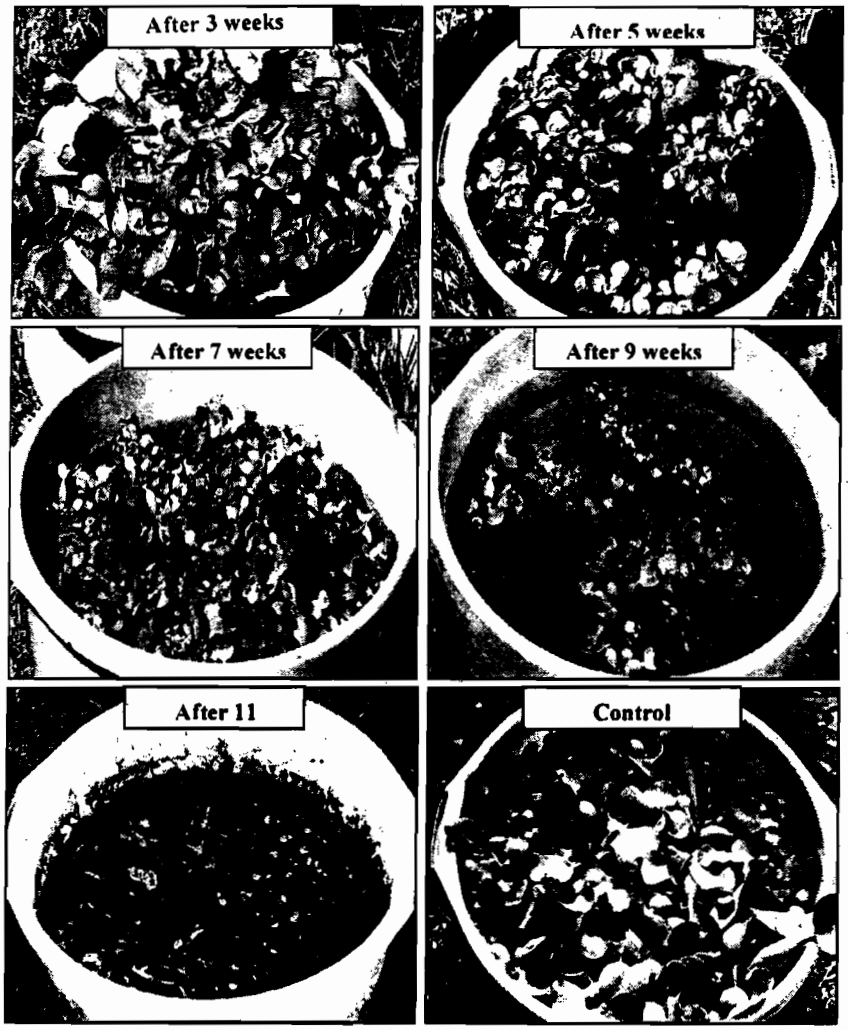


Fig. 2. Performance of Ae5 mycoherbicide (formulated in cottonseed oi emulsion) sprayed on water hyacinth plants on January 23, 2002 ir an outdoor vat study. A 100% weed kill was obtained 11 weeks afte application.

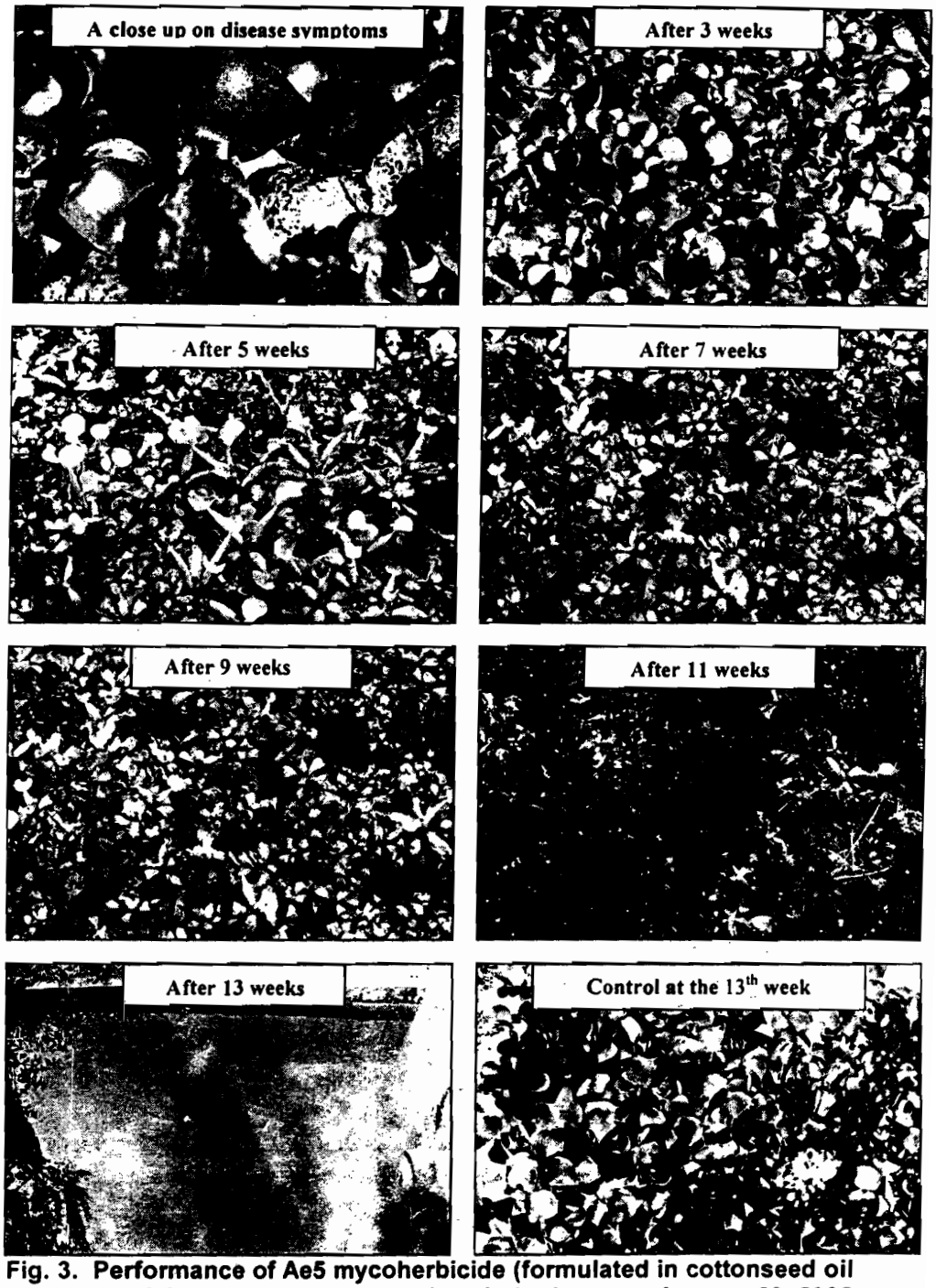


Fig. 3. Performance of Ae5 mycoherbicide (formulated in cottonseed oil emulsion) sprayed on water hyacinth plants on January 23, 2002 in outdoor field-plots.

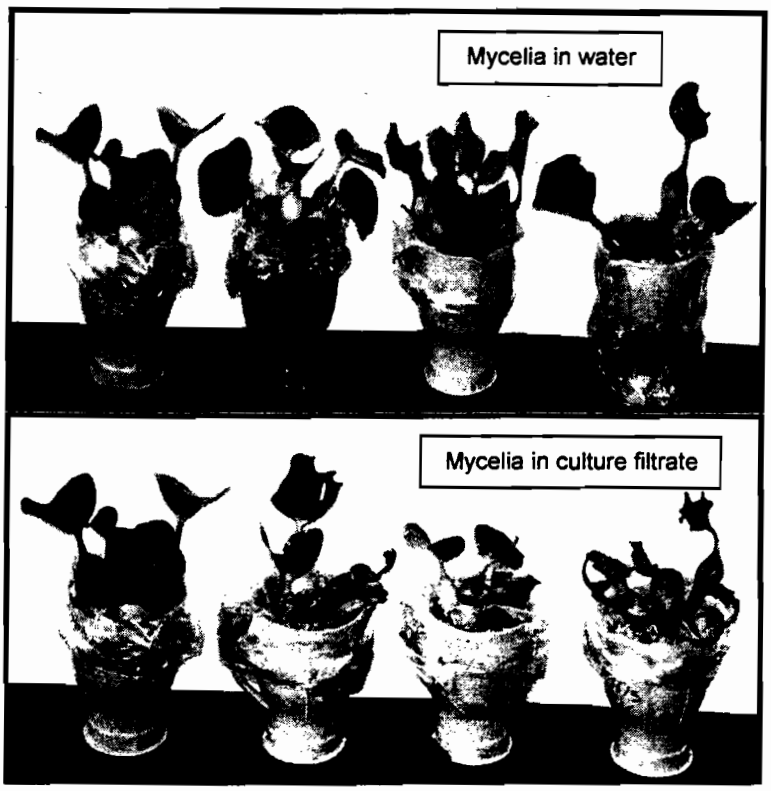
4. Effect of culture age of Ae5 on the inoculum infectivity

The results showed that there was an inverse relationship between the culture age (up to 9 week old) and inoculum infectivity; the younger mycelial inoculum was more virulent on water hyacinth than older ones (Table 6, Fig. 4). There were no significant differences between DS caused by inocula prepared from 9- or 16-week old cultures (Table 6). Inocula containing culture filtrate were more deteriorative than the ones prepared with just water (Fig. 5). Disease symptoms caused by the mycelial suspension in culture filtrate was unlike the symptoms caused by the mycelial suspension in water; the former inoculum caused leaf scorch, which may be due to the deteriorative effect of phytotoxins existing in the culture filtrate, while the latter one produced distinct lesions on plant leaves (Fig. 5).

Table (6): Effect of culture age of *Alternaria eichhorniae* 5 (Ae5) on inoculum infectivity against water hyacinth

Culture age (wk)	Type of inoculum			
	Mycelia in water		Mycelia in culture filtrate	
	Disease incidence (%)	Disease severity (%)	Disease incidence (%)	Disease severity (%)
4	96 a[a]	65 a	96 a	78 a
9	94 a	31 b	96 a	59 b
16	76 a	16 b	84 a	27 b

[a] Values represent means of 5 replicates. Values within a column followed by the same letter are not significantly different according to Tukey's studentized range test ($P = 0.05$).



Control | **Inoculum obtained from 16 week-old culture** | **Inoculum obtained from 9 week-old culture** | **Inoculum obtained from 4 week-old culture**

Fig. 4. Effect of culture age of Ae5 on its inoculum infectivity against water hyacinth plants.

Mycelia in water Mycelia in culture filtrate

Fig. 5. The disease severity caused by Ae5 inocula (from 4-wk old culture) containing or not the culture filtrate of the fungus. Notice that inoculum in culture filtrate is more deteriorative than inoculum in water. Observe the difference in the symptoms; the inoculum in culture filtrate causes leaf scorch while the inoculum in water produces distinct lesions on plant leaves.

5. Effect of the oil content in the Ae5 emulsion bioherbicide on its infectivity against water hyacinth

Reducing the oil content in the Ae5 emulsion formulation from 30 to 5% had no significant effect on the formulation's infectivity against water hyacinth (Table 7). Fungal inocula applied in the aqueous carrier alone (0% oil), while dew was provided immediately after treatment, caused the same levels of DI and DS as they caused by the inocula applied in oil emulsion (at all oil concentrations) (Table 7). However, in the absence of dew, the DI and DS observed on plants treated with fungal inoculum in the aqueous carrier alone were significantly lower than those caused by the inocula applied in oil emulsion (at all oil concentrations) (Table 7).

Table (7): Effect of cottonseed oil concentration in the *Alternaria eichhorniae* 5 (Ae5) emulsion formulation on its bioherbicidal efficacy against water hyacinth

Oil conc. (%) in the final formulation	Disease incidence (%)	Disease severity (%)
0 (Check I)[a]	26.7 c[d]	5 c
0 (Check II)[b]	71.7 b	33.3 b
0 (Check III)[c]	100 a	98.3 a
5	100 a	83.3 a
10	100 a	85 a
15	100 a	90 a
20	100 a	91.7 a
25	100 a	93.3 a
30	100 a	100 a

[a] Fungus-free culture filtrate
[b] Fungus in culture filtrate without dew
[c] Fungus in culture filtrate + dew
[d] Values within a column followed by the same letter are not significantly different according to LS means test ($P \leq 0.001$ for DI and < 0.04 for DS).

DISCUSSION

The bioherbicidal effects of pathogens can be measured as reduced growth rate (fresh-weight yield) of the host plant, fewer living leaves and more dead leaves on individual plants, and lower values of other growth components (Charudattan *et al.*, 1985). Also, water hyacinth can cause substantial water loss due to the combined effects of evaporation and transpiration and thus lessen the usefulness of water impoundments (Moursi, 1976; Pieterse and Murphy, 1990, Shabana *et al.*, 1995b; and Zahran, 1976). Therefore, the biological control efficacy of Ae5 formulations was measured in the present investigation on the basis of their ability to reduce the growth rate and biomass of water hyacinth and to mitigate the water loss from plots. In each case, the cottonseed oil formulation was highly effective. The finding that the high the DS the less water loss, could be attributed to that the ability of infected or dead leaves to make evapotranspiration is much less than the ability of the healthy leaves.

Formulation is key to assuring the efficacy of bioherbicidal agents (Boyette *et al.*, 1991). Formulations such as a suspension, wettable powder, flowable granule, dust, etc., may contain diluents, spreaders, stickers, emulsions, and other inert ingredients that increase a biological control agent's efficacy (Connick *et al.*, 1990, 1991). A major obstacle in the use of foliar fungi for consistent control of aquatic weeds is the need for free moisture (usually dew) for fungal inoculum to germinate and infect the weed. Formulation of Ae5 in invert emulsions or in vegetable oil emulsions enabled the pathogen to infect the weed in the absence of dew. All the oil treatment induced higher levels of disease on water hyacinth plants in comparison with the aqueous suspension in the absence of dew. Similar results were reported by Auld (1993), who examined the use of vegetable oil emulsions to formulate the fungus

Colletotrichum orbiculare for controlling the weed *Xanthium spinosum*, and by Daigle *et al.* (1990) who used the invert emulsion formulations for *Alternaria cassiae* to control sicklepod, a serious weed problem in soybean, cotton, and peanut (Walker and Boyette, 1985). The emulsions contain some relatively pure components (i.e., paraffin wax and mineral oil), but they also contain crude biologicals (i.e., commercial vegetable oils as well as soybean lecithin, a waste byproduct of food oil manufacture). The biologicals may contain components that affect infectivity, not just control free water around the fungal inoculum (Amsellem *et al.*, 1990) which may interpret in part the differences in DS obtained by different emulsion formulations. The evaporation-retarding property of the invert emulsions (Shabana, 1997a) is probably responsible for the results observed on the fungus-inoculated water hyacinth plants in the absence of dew. Although the vegetable oil-based formulations do not have the markedly reduced water evaporation characteristics of an invert emulsion, they impart advantages that may include better wetting characteristics on leaf surface, protection of fungal propagules, and some water retention (Auld, 1993 and Abbas *et al.*, 1996). The precise role of the oil in these emulsion formulations was explained by Greaves *et al.* (1995) who investigated the plant-oil emulsion interaction using transmission electron microscopy and cryo-stereo electron microscopy. Their examinations have revealed that the oil phase of the emulsion rapidly penetrated the intercellular spaces of the field pansy (*Viola arvensis*) leaf tissues and the water diffused from neighboring cells into the oil as microdrops forming an invert emulsion (water-in-oil). The water drops differed throughout the oil phase, reaching the oil film on the leaf surface and, thus, providing as easily available, and continuously supplied, source of water for microbial herbicide development. It is postulated that this mechanism underlies the reduction in dew period requirement measured with the vegetable oil emulsion formulation. Research with insect pathogenic fungi has also manifested improved infectivity with oil formulations (Prior *et al.*, 1988). Our results showed that reducing the oil content in the Ae5 emulsion formulation from 30 to 5% had no effect on the formulation's infectivity against water hyacinth. Thus, high oil content would add costs to the mycoherbicidal formulation and therefore would be uneconomical and unrealistic to apply high oil concentration above that required for field efficacy; accordingly, a level of 5 % of cottonseed oil is regarded as sufficient.

Although the invert emulsions and the vegetable oil emulsions caused high levels of DS on the target weed, the vegetable origin of the oil and its lower content in the formulation in addition to the easiness of preparation and application offer economic and practical advantages over invert emulsions.

In our study, an increase in DS was obtained on water hyacinth when the inoculum concentration was increased up to 10% mycelial inoculum and then remained at the same DS level up to 25%. A high inoculum concentration should give a better distribution of inoculum on plant surfaces and therefore, induces a higher initial levels of disease from which successive cycles of infection can develop (Charudattan, 1988 and Van der Plank, 1975). Regardless, it would be impractical and uneconomical to use very high inoculum levels beyond the optimum for field studies; accordingly a level of 5 to 10% of mycelium is regarded as sufficient. This rate may be

reduced by suitable formulations that provide excellent dispersal characteristics and good coverage over plant foliage.

For the 1998-season trials conducted in outdoor field plots, a 100% control of water hyacinth plants has occurred within 7 weeks after application, while the same level of control (100%) was obtained 13 weeks post treatment in identical plots used in the trial conducted in 2002. This difference in the results between the two seasons may be due either to the change in the time of application suggesting that the weather at mid November could be more suitable for the mycoherbicide to infect the weed and develop weed kill more effectively, or the recovery of Ae5 virulence (which has been lost due to the increased numbers of subculturing during a 3-year period of preservation) was not fully attained in the inoculum used for the 2002 trials.

It was reported by Shabana *et al.* (2001a) that a higher concentration of the partially purified culture extract (10%) of Ae5 was required for symptom expression on water hyacinth leaves suggesting a role of toxins in disease development. Additionally, Shabana (1992) suggested the possibility that many toxins were produced by this fungus that might have involvement in the pathogenicity of the organism. Our finding in the present study that mycelium plus filtrate caused a higher level of DS than mycelium plus water is then logical since in the inoculum of mycelium plus culture filtrate, the fungus was supported with more phytotoxins, those in the culture filtrate which assist in disease initiation and the toxins that the fungus excretes during the infection process, whereas the inoculum of the mycelium in water would only benefit from toxins excreted during pathogenesis.

CONCLUSIONS

The present study suggests that the biocontrol fungus, Ae5, be formulated in cottonseed oil emulsion in order to bypass dew and to induce high DS and weed kill without even exposure to the dew. Cottonseed oil emulsion, which is readily available in the marketplace, offers an effective, easy-to-use option as application formulation for the mycoherbicide Ae5 in the field since it maintains virulence of the fungus in dew-free conditions. However, research is essential to develop a sustainable solution to the water hyacinth problem. Given the highly varied nature and extent of water hyacinth problem worldwide, it is imperative that research on integrated control be undertaken. The emphasis should be on applied answers to the country specific needs.

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استخدام المستحلبات الزيتية لتحسين كفاءة الفطر ألترناريا أيكورنيا كمبيد حيوى لورد النيل

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عزل الفطر ألترناريا أيكورنيا *Alternaria eichhorniae* من نباتات ورد النيل *Eichhornia crassipes* المصابة طبيعيا فى المجارى المائية بدلتا النيل فى مصر وثبت أن لهذا الفطر قدرة مرضية عالية على هذه النباتات. وقد أجريت دراسة لتحديد المدى العوائلى Host range لهذا الفطر باستخدام ٩٧ نوع وصنف نباتى من المحاصيل الاقتصادية الهامة المنزرعة فى مصر وثبت تخصصه على نبات ورد النيل فقط وعدم إصابته لأى من المحاصيل الاقتصادية المختبرة.

وكانت العقبة الرئيسية أمام التطبيق الحقلى الواسع لاستخدام هذا الفطر فى المقاومة البيولوجية لورد النيل هى احتياجه لوجود الماء الحر على سطح النبات لفترة تصل إلى ١٠ ساعات متصلة لينبت الفطر ويخترق البشرة وهذا ما لا يتوفر عادة فى ظروف البيئة الطبيعية ، وبالرغم من المحاولات التى أجريت لتجهيز هذا الفطر كمبيد حيوى بدمجه فى عدد من المواد الغروية المحبة للماء Hydrophilic gels والتى أدت بالفعل إلى زيادة كفاءته تحت ظروف البيئة الطبيعية إلا أنها كانت زيادة محدودة ولهذا فقد أجرى هذا البحث بهدف محاولة التغلب على هذه العقبة عن طريق تجهيز الفطر فيما يسمى بالمستحلبات المعكوسة Invert emulsions أو المستحلبات الزيتية Vegetable oil emulsions والتى فيها يغلف الفطر ومعه جزيئات من الماء بطبقة زيتية تحد من فقد الماء من المبيد الحيوى بالبخر وبالتالى يقل احتياج الفطر لفترة طويلة من الندى واللازمة لإنبات جراثيمه ثم إحداث الإصابة. وقد أوضحت نتائج هذا البحث نجاح هذه الطريقة فى رفع كفاءة المبيد الحيوى لمقاومة نبات ورد النيل حتى فى ظروف الجفاف النسبى وفى غياب الندى. وقد تم اختبار عدد كبير من المستحلبات المعكوسة والزيتية تحت ظروف البيئة الطبيعية وأوضحت النتائج أن جميع المستحلبات المعكوسة والزيتية قد رفعت كفاءة المبيد الحيوى فى إحداث الإصابة وبدرجة معنوية مقارنة بالفطر المعلق فى الماء فقط. وقد أظهر مستحلب الفطر فى زيت بذرة القطن تفوقه على باقى المستحلبات فى مقاومة ورد النيل حيث أدى إلى موت كامل للنباتات (١٠٠% مقاومة) فى فترة زمنية تراوحت بين ٧ – ١٣ أسبوع من بدء المعاملة وذلك تحت ظروف البيئة الطبيعية. وقد كان تركيز اللقاح الأمثل من الفطر هو ١٠% (١٠ جم ميسيلوم طازج فى ١٠٠ ملل مستحلب) ، ومن ناحية أخرى فقد وجد أن المزرعة الحديثة أكثر كفاءة من المسنة فى تأثيرها المرضى، حيث وجد أن اللقاح الفطرى الناتج من مزرعة عمرها ٤ أسابيع أكثر كفاءة فى إحداث المرض على نباتات ورد النيل مقارنة بلقاحات مزارع عمرها ٩ و ١٦ أسبوعا.