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IMPACT EVALUATION OF *NEOCHETINA* SPP. ON DIFFERENT GROWTH STAGES OF WATERHYACINTH

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Abstract: An attempt was made to optimize the minimum required inoculation load of the weevils on three growth stages of waterhyacinth, based on reduction of fresh biomass, number of leaves and ramifications. The small growth stage was controlled earlier than the waterhyacinth of middle growth stage, corresponding to the increase in number of weevils per plant. The large plants could not be controlled even with the inoculation pressure of 20 weevils per plant because of high growth rate. This study suggests that *Neochetina* spp. has the potential to keep the population of the macrophyte at a sub-economic density, through a basic inoculation load of weevils in due course of time.

Key words: *Eichhornia crassipes,* feeding scars, *Neochetina* spp., waterhyacinth, weevils

INTRODUCTION

The exotic waterhyacinth weevils, *Neochetina bruchi* Hastache and *N. eichhorniae* Warner are reported to be the most effective and widely used biocontrol agents of waterhyacinth and have contributed to the control of this weed in a number of countries (Jayanth 1988; Harley 1990; Center 1994; Corodo 1999; Julien, 2001). In India, *N. bruchi* and *N. eichhorniae* coexist on waterhyacinth in the same habitat in about 1:0.04 ratio (unpublished data). Spectacular success has been achieved at Hebbal tank in Bangalore (India) causing 95% control within a span of two years (Jayanth 1988), Loktak lake in Manipur (Jayanth and Visalakshi 1989) and several ponds in Jabalpur (Sushilkumar and Bhan 1997). However, there were several instances where weevil releases have been a total failure, for example Kengeri tank in Bangalore (PDBC 1994).

The establishment of biocontrol agents and the control of weed depend on the interactions with their host plant and the environment where they are released. There are situations where these insects either do not establish, establish but do not provide a complete control or desired level of the control. Several limitations of the *Neochetina* weevils in controlling waterhyacinth have been recognized by some workers (Perkins 1973, 1978; DeLoach and Corodo 1976). One of the important limitations is the lack of knowledge of number of weevils required to control a particular number and growth stages of waterhyacinth in a given area. Researches in Argentina, USA and Australia have indicated that plant quality affects the life history (Center and Durden 1986; Wright 1984) and biocontrol potential of the weevils. Such limitations of the weevils create suitable conditions for waterhyacinth to reproduce at a higher rate than the weevil's population growth.

Moreover, several authors (Cooley and Martin 1978; Gopal 1987) have distinguished 3 biotypes of waterhyacinth on the basis of differences in leaf size as 'small', 'medium' and 'large' (or super hyacinth) which differed in tolerance to the attack by *Neochetina* spp. Thus the number of weevils required to control these three types of growth stages would also differ. Kannan and Kathiresan (1999) reported varied numbers of weevils required to control different growth stages of waterhyacinth. Their results were based on the experiment they did in a very small basin where the chances of waterhyacinth growth was restricted in want of proper space to grow and proliferate. Keeping in view the above facts, this experiment was conducted in large water tanks, to determine the weevil density required to control waterhyacinth at different growth stages.

MATERIALS AND METHODS

Inoculation load of *Neochetina* spp. on different growth stages of waterhyacinth

Healthy and uninfested waterhyacinth of different growth stages were collected from local ponds and brought to aquatic experimental site at our research farm. In order to free the plants from any possible insect infestation they were sprayed with carbaryl 50 wp (2.5 g/l) and left for three days. They were rewashed and released in experimental tanks (volume 0.57 cu m and exposed surface area of 0.64 m^2). For each of the three growth stages of waterhyacinth, five different inoculation loads of the weevils (4, 8, 12, 16 and 20 per plant) were released. These

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weevils were collected from waterhyacinth infested water bodies in Jabalpur. The weevils were released without segregating them into two species as they naturally co-exist on waterhyacinth under field conditions. Each treatment had three replications and each replication had five plants. Tanks with plants receiving no weevil release were taken as control. The control tanks were treated with monocrotophos (0.5%) at a monthly interval to check the attack of weevils from nearby tanks. The tanks were regularly filled with water and fertilized with farmyard manure as and when required for the healthy growth of waterhyacinth.

Observations were performed at intervals of 10 days till the death of the plants. The control plants of each of the growth stages were monitored until the death of the last weevil infested plant of the respective growth stage. Each waterhyacinth plant was observed for the following parameters: percentage of damage, number of green leaves (live), fresh weight, number of feeding scars made by the weevils, number of ramifications (daughter plants).

Effect of surface area on the effectiveness of waterhyacinth weevils was also compared by releasing the same number of inoculation loads of weevils on waterhyacinth in small water tub having the volume of 50 litres and exposed surface area of 0.2 m^2.

Feeding behaviour of the *Neochetina* spp.

Feeding behaviour of the waterhyacinth weevils was also observed on five plants of small, medium and large growth stages. The three growth stages were kept separately in tubs filled with water. On each plant, 2 pairs of weevils were released. After three days all the scars on either surface of the leaf that were readily identifiable were counted. Compound scars were separated into component scars by noting any obvious discontinuities in the normally regular scar borders or on the differential depth of feeding. Feeding scars were counted by marking each counted scar using a marker pen. Marking of each scar prevented accidental recounting.

Statistical analysis

The experiment was performed in completely randomized design (CRD) with three replicates. The test results were subjected to analysis of variance (ANOVA) using statistical software, Genstat. The ratio of mean sum of squares of treatments and errors i.e., 'F' ratio was tested at 5% level of significance. Further, treatment means were compared with least significant difference (LSD) at 5% level of significance. Averages taken from all plants examined in each sampling unit were used in each analysis.

RESULTS

Effect of different inoculation load of *Neochetina* spp. on different growth stages of waterhyacinth

The control plants at all growth stages were large, robust and healthy with no weevil attack. *Neochetina* spp. had a significant impact on waterhyacinth plant vigor and reproduction. The number of days taken (Table 1) to completely drown waterhyacinth of three growth stages, differed with different inoculation loads of the weevils. The damage percentage (Fig. 1), fresh biomass (Fig. 2), number of live leaves (Fig. 3) and number of daughter plants or ramifications (Fig. 4) were adversely affected by weevil infestation and varied from stage to stage. The grubs produced by the released weevils also caused severe damage by tunneling through the petiole.

Table 1. Number of days taken by *Neochetina* spp. at different inoculation load to control waterhyacinth at different growth stages

Inoculation load of weevils/ plant	Days taken to control growth stages		
	small	medium	large
4	50	70	no control
8	40	60	no control
12	10	50	no control
16	10	40	no control
20	10	20	no control

Small growth stage. Small growth stage of waterhyacinth was controlled rapidly compared to their corresponding middle and large growth stages. There was an increase in damage percentage corresponding to the increase in weevil load. By the 10th day ($F = 172.39$, $p < 0.01$) a highly significant difference in percentage damage was obtained between the control tanks (with no damage), and the treated tanks with 4 (11.7% damage), 8 (47% damage), 12 (96% damage), 16 (96% damage) and 20 weevils per plant (95% damage). By 20th day ($F = 608.77$, $p < 0.01$) 100% damage was caused by 16 and 20 weevils. 12 weevils caused 100% damage by day 30 ($F = 859.75$, $p < 0.01$). 8 and 4 weevils could cause 100% damage by 40 days ($F = 3360.39$, $p < 0.01$) and 50 days ($F = 20982.76$, $p < 0.01$) respectively. Similar observations were obtained for fresh biomass, number of leaves and ramifications as well. The number of feeding scars was more on small growth stage of waterhyacinth as compared to equal number of weevils released on middle or large growth stage proving the preferential feeding of the weevils on younger leaves.

In another experiment carried out in small water containers of 50 litres capacity, the time taken to kill the weed was reduced to nearly half as compared to the large tank conditions (Table 2). In small tubs, 4 and 8 weevils could control small growth stage waterhyacinth in 25 and 15 days, respectively, while 12, 16 and 20 weevils could drown the weed mat in 20, 15 and 10 days, respectively.

Table 2. Effect of surface area on biological control of waterhyacinth by *Neochetina* spp.

Inoculation load of weevils/ plant	Days taken to control water hyacinth	
	large tank (surface area of 0.6 m^2)	small bucket (surface area of 0.2 m^2)
4	50	25
8	40	25
12	30	20
16	20	15
20	20	10
Control	no control	no control

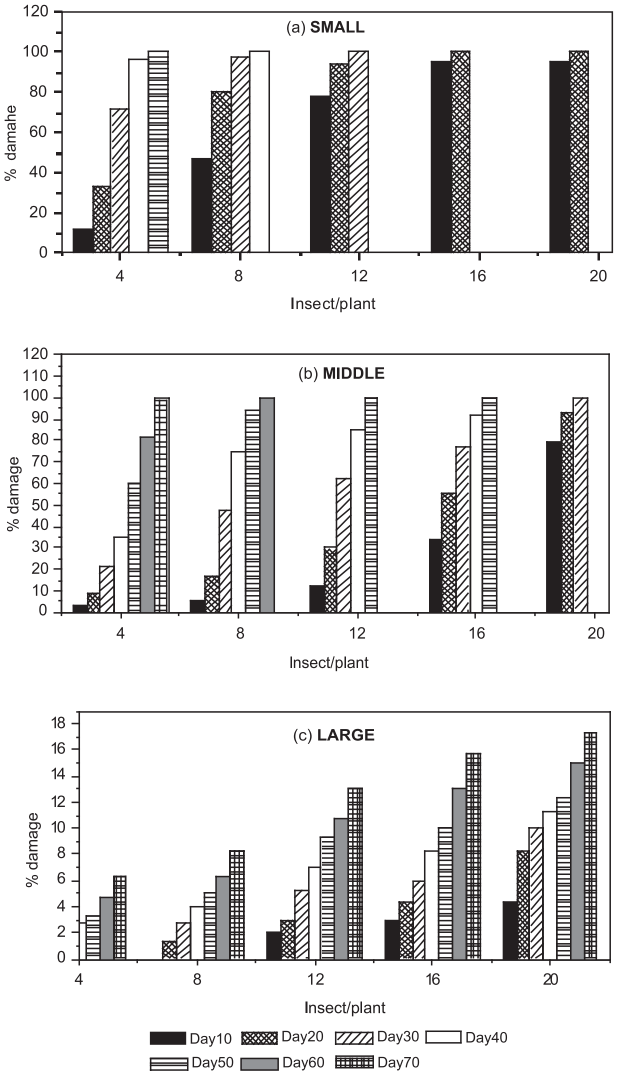

Fig. 1. Mean per cent damage caused by *Neochetina* spp. of different inoculation load to control waterhyacinth at different growth stages during different days after release. (a) small growth stage, (b) medium growth stage, (c) large growth stage

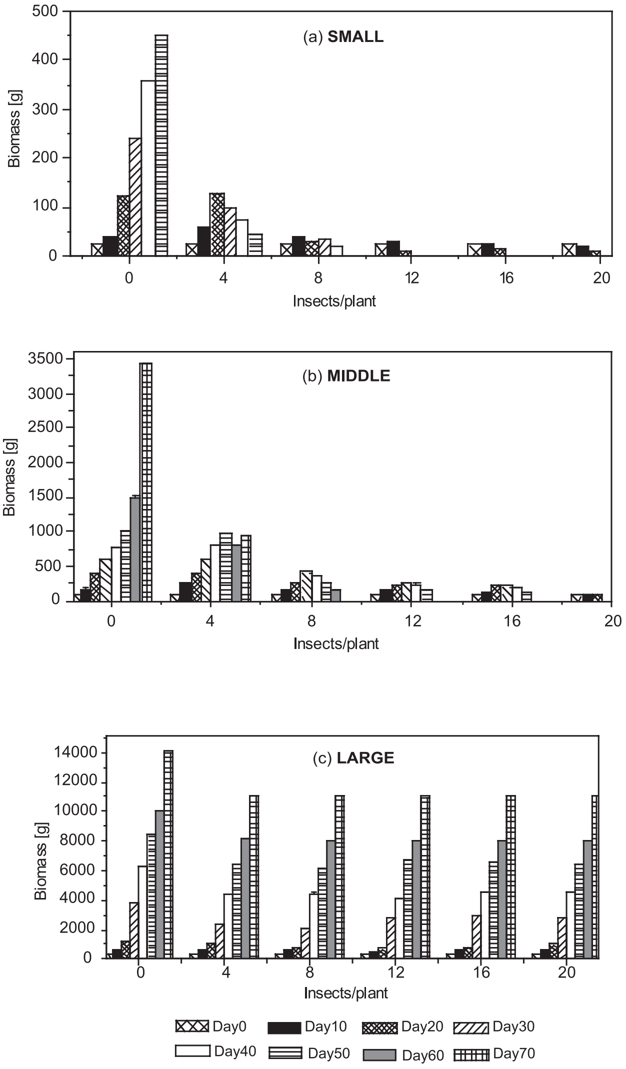

Fig. 2. Impact of *Neochetina* spp. of different inoculation load on average wet biomass of waterhyacinth at different growth stages at different days after release. (a) small growth stage, (b) medium growth stage, (c) large growth stage

Medium growth stage. A longer time was necessary for the weevils to control middle growth stage as compared to the small growth stage plants. There was significant difference in the percentage damage (Fig. 1) caused by the weevils at different inoculation loads by the 10th day ($F = 363.05$, $p < 0.01$). Four weevils took 70 days ($F = 3608.53$, $p < 0.01$) to cause 100% damage while 8, 12, 16 and 20 weevils took 60 ($F = 4074.66$, $p < 0.01$), 50 ($F = 3520.5$, $p < 0.01$), 40 ($F = 720.54$, $p < 0.01$) and 20 days ($F = 273.18$, $p < 0.01$), respectively (Table 1). Similarly biomass of the waterhyacinth was also significantly influenced by different inoculation loads of the waterhyacinth weevils (Fig. 2). There was no significant difference in leaf production (Fig. 3) in plants with different inoculation loads by 10th and 20th day, which was abridged by 30 days ($F = 36.94$, $p < 0.01$). The leaves produced were comparatively smaller in size as compared to the control plants. There was also an increase in root density with the increase in density of weevils per plants. Though by the 10th day ($F = 20.75$, $p < 0.01$) there was no significant difference in the number of ramifications in control tank and treatment with 4 and 8 weevils but with the passing of time growth of the ramifications was also significantly checked even by 4 pairs of weevils per plant (Fig. 4) by the 70th day ($F = 13381.83$, $p < 0.01$).

Large growth stage. In the tanks containing large growth stage waterhyacinth, there was hardly any visible damage caused even by the highest inoculation load of the weevils by 10th day. Though feeding scars made by the weevil were visible, the large growth stage waterhyacinth could not be controlled even at the highest inoculation load of 20 weevils per plant (Table 1). Fresh biomass of waterhyacinth in control tanks was significantly high by 20th day ($F = 63.47$, $p < 0.01$) as compared to treated tanks. Similar trend was seen on 70th day ($F = 332.34$, $p < 0.01$) as well. Yet by day 70 ($F = 70.22$, $p < 0.01$) there was hardly 17% damage caused by the weevils at inoculation load of 20 weevils per plant, which was easily recovered by the high growth rate of the plants. There was no significant

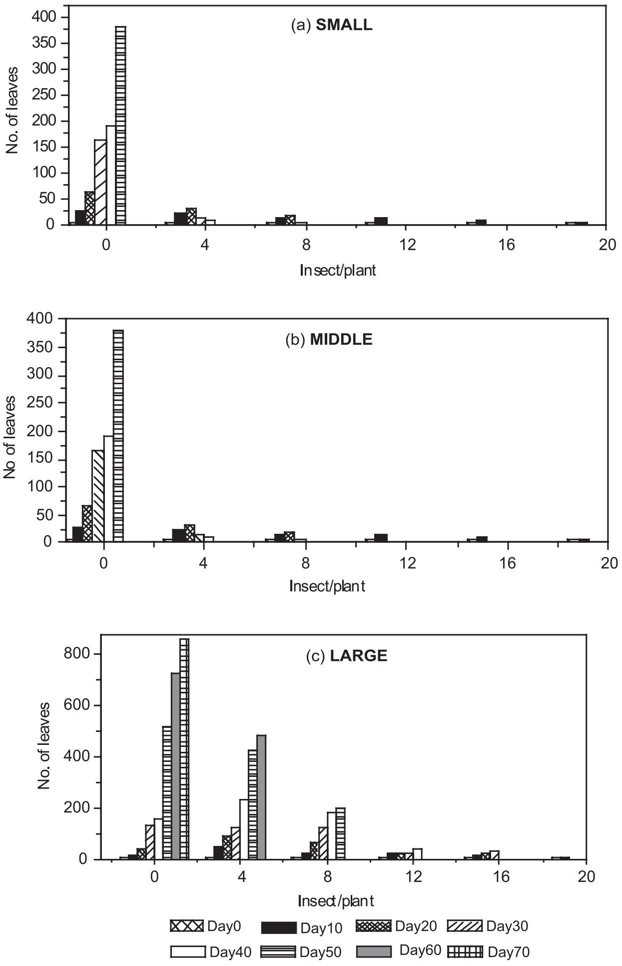

Fig. 3. Impact of *Neochetina* spp. of different inoculation load on average number of leaves of waterhyacinth (a) small growth stage, (b) medium growth stage, (c) large growth stage, at different days after release

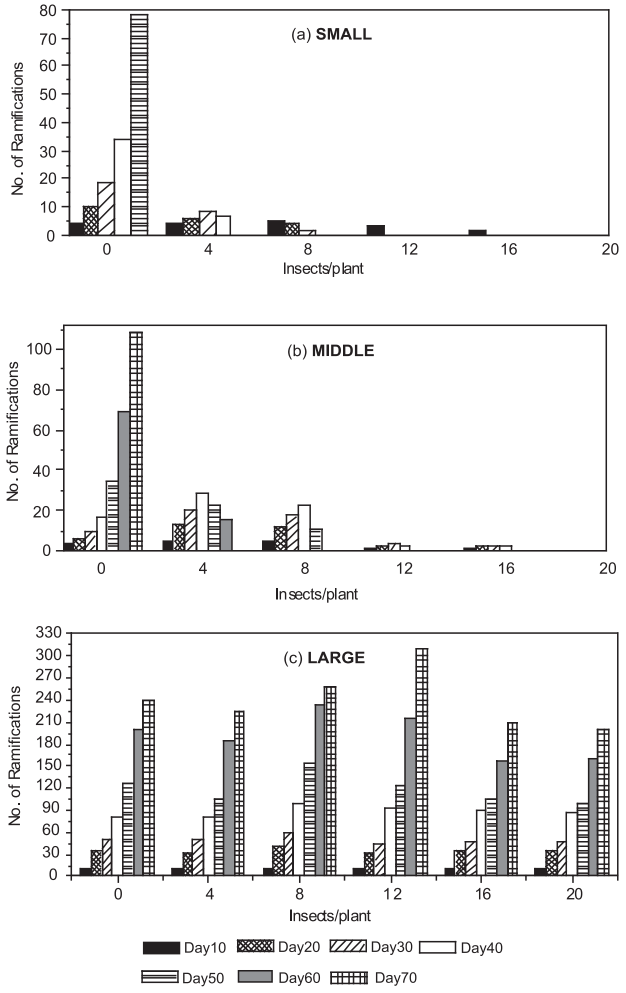

Fig. 4. Impact of *Neochetina* spp. of different inoculation load on number of ramifications of waterhyacinth at different growth stages. (a) small growth stage, (b) medium growth stage, (c) large growth stage, at different days after release

difference between the percentage damage between treatments of 20 and 16 weevils but there was a significant difference between treatments 12, 8, 4 weevils per plant and the control.

By the 10th day ($F = 12.36$, $p < 0.01$) the number of leaves in tanks with large growth stage was significantly higher in control tank as compared to weevil inoculated tanks. By 70th day ($F = 3.72$, $p > 0.05$), there was no significant difference between the control tanks and the tanks with inoculation of 4, 8 and 12 weevil per plant. Further there was no significant difference in the number of leaves in tanks with the release of 8, 12, 16 and 20 weevils per plant. Similarly no significant trend could be obtained for rapid increase in the number of ramifications in the tanks containing large growth staged waterhyacinth.

Feeding behaviour of the waterhyacinth weevils

By feeding on the epidermal tissues of the laminae and petiole and removing the cuticle and part of the mesophyll tissue, *Neochetina* spp. caused the characteristic feeding scars. The weevils preferred to feed on lower surface and upper petiole of the leaf and responded to the quality of waterhyacinth (Fig. 5). The weevils preferred younger leaves than the older ones. Feeding declined with direct proportion to the leaf growth stage. They fed preferentially on the soft tissue of unfurled young lamina and upper portions of young petiole. In casual observation, the weevils, when given choice among the three growth stages, congregated in the centre most folded leaf of the small growth stages. On the small growth staged waterhyacinth number of feeding scars after 3 days ($F = 29.05$, $p < 0.001$) was highly significant as compared to other treatments. Similar observations were obtained for middle ($F = 35.32$, $p < 0.001$) and large growth stages ($F = 13.65$, $p < 0.001$) after 3 days of feeding. By 10th day also highly significant difference was noticed in the number of scars by 20 weevils as compared to other treatments for small growth stage ($F = 12.25$, $p < 0.001$) while 4, 8 and 12 weevils were on par with each other. For middle ($F = 38.26$, $p < 0.001$) and large ($F = 27.91$, $p < 0.001$) growth stages also similar observations were obtained on 10th day. Feeding scars couldn't be counted after day 10, especially on small growth stage plants as the scars were too dense and undifferentiated particularly at the leaf isthmus.

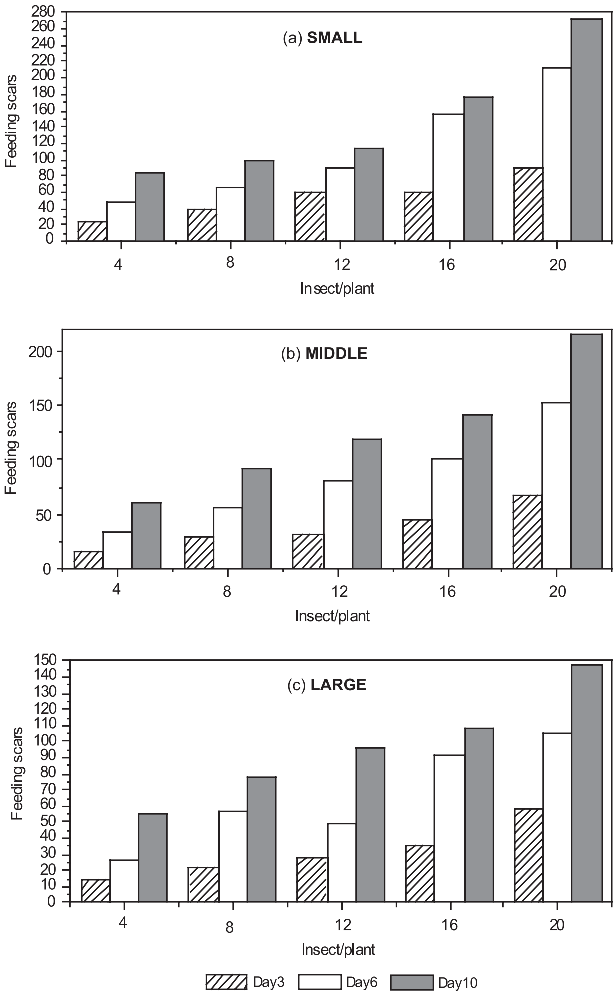

Fig. 5. Feeding scars by *Neochetina* spp. on different growth stages of waterhyacinth. (a) small growth stage, (b) medium growth stage, (c)large growth stage

DISCUSSION

Neochetina spp. can definitely provide substantial control of waterhyacinth with a basic inoculation load of weevils but at different time intervals. Though Visalakshy and Jayanth (1995) reported that 4.4 weevils per plant could bring about a collapse of the waterhyacinth mat but growth stage and time period required for the action was not mentioned. Contrary to this El Abjar (1981) reported 50% reduction in waterhyacinth population in 4 months but did not mention the inoculation load of the weevil implemented. Aguilar *et al.* (2003) reported that the density of adult weevils required to attain control was about 6 weevils per plant based on both the field and cage trials but they didn't study plant size in relation to weevil population and time duration required to bring about this control. Studies by Forno (1981) under glasshouse conditions in Australia showed that 10 pairs of *N. eichhorniae* and their larval progeny per 0.58 m^2 area reduced the plant growth of floating, anchored and rooted plant forms within one insect generation. Without mentioning growth stage, Center and Durden (1986) reported that under field conditions in Florida weevil attack reduced the plant size and also live waterhyacinth plant and their shoot size. Period ranging from 14 months to 6 years were found to be required for obtaining significant level of control by different authors after inoculation of the bioagent in different aquatic body (Groyer and Stark 1984; DeLoach and Cordo 1983; El Abjar 1981). In Australia, Wright (1979) reported the collapse of a waterhyacinth population beginning within 2 years after release of *N. eichhorniae*. Groyer and Stark (1984) showed significant control by *N. eichhorniae* within 14 months at a site in Louisiana. DeLoach and Cordo (1983) obtained 67% control of waterhyacinth in Argentina by releases of *N. bruchi* within 4 months and 90–95% control in 6 years. Goyer and Stark (1981) observed that 5 adults could kill a medium sized plant in 10 days under laboratory conditions while in our experiment the medium size plant were killed in 20 days by inoculation load of 20 weevils. Early control by the weevils in Goyer and Stark's (1981) experiment may be due to keeping of waterhyacinth plants in limited space which might have restricted the growth and proliferation of waterhyacinth. In our experiment, waterhyacinth plants were kept in large size tanks in limited number due to which they could get ample space to grow and proliferate. The same experiment done in small tubs could kill waterhyacinth of the same growth stage in nearly half time. This was mainly because of less volume of water and less surface area for the waterhyacinth to proliferate. It shows that surface area present for the weed's growth does affect the control potential of the weevils. This is the reason that in natural conditions especially in nutrient rich water bodies, the weevils may take from 2 to 6 years to cause significant level of control.

The weevils definitely slowed down plant growth and reduced waterhyacinth density even in large growth stage where no complete control could be obtained even at highest inoculation load. Although higher weevil population increased production of smaller leaf but more leaves were killed due to higher weevil density. This indicates that large growth stage may take longer time to be controlled by the *Neochetina* spp. in large water bodies. Therefore high inoculation load of the weevils should be released for biological control of large growth stage waterhyacinth.

The success or failure of a biocontrol agent to establish itself depends partially on the health of the biocontrol agents released and on the weed physiology and the nutrient level of the water body. Under natural conditions, it has been observed that despite of high weevil population there was no substantial reduction in plant size, wet biomass etc, while in the water tubs or tank condition for the control was much efficient. Center and Wright (1991) suggested that plant quality might influence the abundance of *Neochetina* spp. and hence the control of waterhyacinth. It was seen that higher quality plants could be controlled faster than the poor quality plants and young plants were controlled earlier than the older ones. They found that adult of *N. eichhorniae* are attracted to young leaves because of presence of some volatile substance that stimulate them to feed especially at previous site of injury. There may be a decline in the attractant volatile substance with increase in age of the plant.

Under natural conditions, time period to control the same growth stage of waterhyacinth by different inoculation load of weevils may be many folds higher than in our experimental conditions. Due to eutropic conditions of the water bodies plant growth and reproduction occurs at a faster rate than the weevils to inflict substantial control. Thus though the present work concentrates on inoculation load of the weevils and plant growth stage, there are other factors that have tremendous impact on waterhyacinth management which needs to be taken into consideration before implementing the biological control of this majestic macrophyte in large lakes and ponds.

This information on inoculation load may be utilized in introductory release of weevils to control waterhyacinth. This study suggests that large number of weevils should be released in different patches on large growth stage waterhyacinth to bring about effective and quicker control while inoculation load can be reduced accordingly for middle or smaller growth stages.

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POLISH SUMMARY

OCENA WPŁYWU *NEOCHETINA* SPP. NA RÓŻNE STADIA ROZWOJOWE *EICHHORNIA CRASSIPES*

Podjęto próbę określenie optymalnej liczby chrząszczy *Neochhetina* spp., użytego do zwalczania *Eichhornia crassipes* w trzech fazach rozwojowych roślin biorąc pod uwagę: świeżą biomasę, liczbę liści i rozgałęzień. Zwalczanie *E. crassipes* we wczesnych fazach rozwoju następowało wcześniej niż zwalczanie roślin będących pełni rozwoju, co odpowiadało zwiększeniu liczby chrząszczy na roślinie. Dużych roślin nie można było zwalczyć nawet w przypadku wysokiego nasilenia występowania, wynoszącego 20 chrząszczy na roślinę z powodu szybkiego tempa wzrostu roślin. Te badania sugerują, że *Neochetia* spp. posiada potencjał utrzymania populacji *E. crassipes* poniżej ekonomicznego poziomu szkodliwości, co związane jest z ilością chrząszczy występujących na przestrzeni czasu.