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RESEARCH ARTICLE

QUANTIFYING THE EFFICIENCY OF THE BEETLE (*GOSTROPHYSAVIRDULA*) AS BIOLOGICAL CONTROL AGENT ON WEEDY PLANT SORREL DOCK (*RUMEX CRISPUS*) AT DIFFERENT SEEDLING GROWTH STAGES

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ABSTRACT

In four glasshouse experiments, efficiency and quantifying using the green dock beetle (Gastrophysa virdula) as biological control agent of sorrel dock (*Rumex crispus*) was studied. The effect of the green dock beetle (Gastrophysa virdula) on *Rumex crispus*was investigated at four levels of beetles number/plant(pot); 0, 1, 2 or 3 beetles/plant (pot) at four different seedlings growth stages of *Rumex crispus* measured by the average of the total leaf area/plant (pot); 1.22 cm2/plant (pot), 4.45 cm2/plant (pot), 11.56cm2/plant (pot) or 71.52cm2 plant (pot).Beetle grazing resulted in a great reduction in *Rumex crispus* dry weight (g)/plant (pot) and shoots number/plant (pot). Statistical analysis showed significant reduction in the average of the four growth stages of *Rumex crispus* dry weight (g)/plant (pot) and shoots number/plant (pot)at high level of significance of ($\leq$0.01) even in the presence of only one beetle/plant (pot). The presence of two or three beetle sometimes increased the reduction although it was not significant and dramatic confirming great effect of one beetle on one seedling of the weed. The present of one beetle reduced the average dry weight of *Rumex crisps* (g)/plant (pot) of the four growth stages by 63%. Whereas, the percentage of reduction in *Rumex crispus* shoot numbers /plant (pot) as a result of the present of one beetle was 49%. Dry weight (g)/plant (pot) and shoots number/plant (pot) of *Rumex crispus* have been affected significantly ($P \leq 0.01$) by the grazing of one beetle at all examined growth stages and this effect continued with the increase of beetle numbers/plant (pot) at the four growth stages. However, the increase in beetle number/plant (pot) was not of significant effect in all examined growth stages of *Rumex crispus* confirming the efficiency of one beetle to create considerable effect on one *Rumex crispus* seedling at all examined four growth stages. After four months from beetle grazing, *Rumex crispus* seedlings were not able to regrow confirming to be dead especially at smaller seedling stages. At the oldest growth stage with average of total leaf area of 71.52/plant (pot), some plants of *Rumex crispus* reemerged. This suggests that the highest beetle effect occurs in early seedling stages of Rumex crispus. Combining beetle grazing with other weed control measure at older growth stages could give better result in suppressing the weed. Statistical analysis showed high correlation (0.77) between the measurements taken for *Rumex crispus* seedlings (dry weight (g)/plant (pot) and shoots number/plant (pot)) at the four seedlings growth stages which confirms similarity of the beetle effect on *Rumex crispus*at all examined growth stages. Moreover, it does show similar effect of the beetle on the two measurements of *Rumex crispus* growth (dry weight (g)/plant (pot) and shoots number/plant (pot)) which indicate that the beetle grazing covers both leaves and tillers of *Rumex crispus*at similar amount including the whole above ground fresh matter of the weed.

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INTRODUCTION

Weedy plants represent a widely distributed plant species and biologically important components of most natural and semi-natural ecosystems in the world. They persist in arable, range lands, forest and aquatic ecosystems (Sher and Alyemeny, 2011). However, they have been defined as unwanted obnoxious plants when growing in areas where they are unwanted. They are nuisance to other plants because of their adverse effects (Chaudhary and Akram, 1987; Strokey, 2006). Weeds became important constraints in agricultural production systems (Williams and West, 2000). They compete with the desirable plants for the available

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essential resources for plant growth which result in a reduced growth and production. The losses in plant growth and crops yield usually result from weeds competition for water, nutrient uptake, light, space and other ecological resources (Hill *et al*, 1977). Weed populations continue to persist in both grass and arable lands despite of repeated application of different control measures. They are the rivals of the other plants and crops as the requirements of both are identical. They need soil, space, light, water, mineral salts (Alyemeny, 1999, Zhao *et al*., 2006; Dunbabin, 2007). Weeds also exhibit allelopathic effects (khanh *et al*., 2005; belz *et al*., 2007; Javaid *et al*., 2007) and depress desirable plants growth by secreting toxic substances from their living decaying parts (Singh *et al*., 2005; Evidente *et al*., 2007). Weeds also reported to act as hosts of diverse plant pests (Oudejan, 1984). The docks and sorrels represent the genus *Rumex* L., consisting of about 200 species of annual, biennial and perennial herbs botanically classified under family Polygonaceae.

Members of Genus *Rumex* are very common perennial herbs growing mainly in the northern hemisphere, but various species have been introduced almost everywhere. Some are nuisance weeds and are sometimes called dock weed, but some are grown for their edible leaves. *Rumex crispus* (curled duck) is among the most common weed species worldwide (Zaller, 2004). *Rumex obtusifolius* is also reported to be one of the five most widely distributed non-cultivated plant species in the world. Both are troublesome weeds in grassland as well as arable lands. They have been adapted to a great range of altitude. *Rumex* species are of great importance from agricultural point view as they compete with sown plants or native pasture species for space which could be utilized by favorite plants (Salt and whittaker, 1998). For example, in Germany, seven out of ten of grasslands farms are heavily infested by *Rumex* species (Bach, 1992; Hofmann, 1992). In Japan 60% of grasslands are reported to be contaminated by *Rumex* species (Hongo, 1986). In Europe, it is estimated that 80% of used herbicides in grasslands farming are applied for *Rumex* species control (Gallery, 1989).

The perennial weed species *Rumex crispus* L., or curled dock, is a major problem in many parts of the world. Its ability to germinate from seed, its persistent taproot system, high capacity of regrowth after cutting and massive seed production which remain viable in the soil for long time all lead to its heavy persistence in agriculture world as well as grassland (Pye,2008). *Rumex crispus* (curled duck) is common weeds of arable lands as well because of its ability to flower number of times though the year and produce large number of seeds which remain viable in the soil for many years and germinate in different environmental conditions with a quick seedling establishment (Cavers and Harper, 1964). In addition, it shows high ability to regrow from root fragments left after cultivation or cutting (Gwynne and Murray, 1985; Pino *et al*., 1995). (Zaller, 2004) synthesized 700 research papers concentrating mainly on the ecology and non-control of *Rumex crispus*. He concluded that although *Rumex crispus* species is among the most frequent studied plant species worldwide, there are still many aspects regarding its ecology and non-chemical control have not been yet addressed.

In ancient times, Chinese found that growing ant populations in their citrus groves decreased populations of large boring beetles and caterpillars. So, using natural enemy to control pest marked the start of biocontrol which became more relevant today. Biological control of weeds involves the use of foreign and native organisms to attack weeds. It includes using living organisms such as insects, nematodes, bacteria, or fungi to reduce weed effects. Weed biological control should provide an environmentally method that complements conventional methods. It should also contribute to the development of weed control strategies since most of the weeds have developed resistance to herbicides. Biological control agents usually target specific weeds. As roots provide plants with water and nutrients, some biocontrol agents could attack roots and stunt plant growth consequently.

Some bacteria live on root surfaces and release toxins that stunt root growth. Some fungi could infect roots and disrupt the water transport systems which reduces leaf growth. Beneficial insects and nematodes feed on the weed roots causing injury which allows bacteria and fungi penetration. Weed leaves produce energy for the plant, Insects feed on weed leaves reduce leaves surface which limit the available energy for the weed and affect its growth. In any case, there will be little energy for weed growth whether though damage on roots or leaves. So, sever infestations of the biocontrol agents reduce weeds adverse effects on wanted plants (Harley and Forno, 1992; Julein and White, 1997). Unlike biological control of insect pests, weed biocontrol has been investigated in the mid-20th century and there have been a recent important cases of success (Martinkova and Honek, 2004).

Gastrophysavirdula (Coleoptera; Chrysomelidae) is an oligophagous herbivore who prefers grazing leaves of dock (*Rumex species*). For its feeding habit, it is considered a biocontrol agent of weedy plants of dock (*Rumex species*). It contributes effectively in suppressing the effect of *Rumex* species plants but it does not control it alone. Combining using the beetle (*Grastrophysavirdula*) as a biocontrol agents with other control measures approved to achieve effective control of *Rumex s*pecies (Martinkovaand Honek, 2004). The green dock leaf beetles are native and widespread in Europe living in predominantly humid habitats. Adult beetles hibernate in the soil at 3-8 cm depth. They emerge and copulate in April/May. Females deposit eggs in groups of about 35 on the abaxial side of the host plants leaves.

Once eclosion occurred, beetle passes three larval stages before pupation to become adults. The life cycle of the beetle is usually influenced by temperature and other environmental conditions. The favorite temperature for the beetle development process is 22-25 C^{0} and the life cycle time is about 24 days. In the laboratory five generation per year can be achieved whereas, only 2-4 generations could occur in the field depending on the habitat. The interest in the biology of the beetle *Gastrophysa virdula* has been increasing with the increase in the potential of using it as a biocontrol agent of *Rumux*species (Dagmar *et al*, 2011). The most thoroughly studied organisms for *Rumex* biocontrol are the Coleoptera; Gastrophysavirdula. Out of 110 studies published to date on biological control of *Rumex* species, 50% of them used insects mainly from the order Coleoptera. *Gastrophysa virdula* have been reported to reduce *Rumex* seed production, regeneration and to alter *Rumex* cover. However, badly attached *Rumex* plants by the beetle *Gastrophysa virdula* were rarely reported to be dead as a consequence (Zaller, 2004). There have been several studies investigating the effect of the beetle; *Gastrophysa virdula* on *Rumex* species growth. However, no attempts were made to quantify the beetle effect. This research work is attempt to quantify the beetle effects on *Rumex crispus* at early growth stages.

MATERIALS AND METHODS

Preparation of *Rumex crispus* seedling populations

Commercial seeds sample of *Rumex crispus* from (Hebiseeds; specialist seed provider in south east U.K) were sown in 3.5 inches pots (250 pots) in the beginning of September 2014 in a glasshouse at school of biological sciences of the University of Reading-UK. Pots were filled with mixed soil(sand, loam and organic matter). One seed/pot was buried near the surface. NPk fertilizer(17%,11%,10%) at level of 25 kg/ha was added to each pot two weeks after sowing to enhance tillers emergence and growth. Plant populations were water regularly. 112pots were selected randomly from the whole population and marked for leaf area measurement. Other pots left for introduction to beetle as will be described below.

Leaf area measurement and calculation

Estimated leaf area was only measured by ruler for seedlings of *Rumex crispus* before introduction to beetles. Estimated leaf area was measured also by ruler for leaves of other 28 plants were selected randomly from *Rumex crispus* seedlings population. Also, actual leaf area was recorded for the same plants using the soft wear (Win DIAS3; Image Analysis System) program. This procedure was repeated prior to each stage of *Rumex crispus* seedlings introduction to beetle in order to determine the actual leaf area for *Rumex crispus* seedlings before beetle introduction. Actual leaf area/plant for *Rumex crispus* seedlings were introduced to beetle at each stage was calculated using liner regression analysis equation;

$$Y = B\ constant + B\ var\ X$$

The actual leaf area of *Rumex crispus* seedlings/plant were introduced to the beetle were then calculated based on liner regression equation at the four stages and were; stage 1:1.22 cm2/plant, stage2:4.45 cm2/plant , stage3:11.56cm2/plant, stage: 71.52cm2.

The Beetle (*Gastrophysa virdula*) culture

Beetle propagation culture started two months prior to *Rumex crispus* seedlings sowing. Leaves of *Rumex obtosifolus* or *Rumex crispus* carrying the beetle (*Gosrtophysa virdula*) eggs were collected from wild forest in the north of wales in the early summer of 2014. Leaf patches of *Rumex obtosifolus* or *Rumex crispus* containing eggs were placed over filter paper in petri dishes in the entomology lab. Filter paper were kept moistened lightly.

Few days later, when larvae were produced, leaf tissues carrying small larvae were moved to plastic container were designed to allow enough light and oxygen through small holes in the top cover of the container. The holes were covered by net to disallow beetle larvae to move out of the containers. Larvae were fed daily with *Rumex obtusifolius* or *Rumex crispus* fresh leaves were collected from *Rumex* plants were growing in grasslands around the campus and nearby gardens. Once larvae moved to pupa stage and became adults, they were moved to other containers and were fed daily while leaves patches carrying eggs were taken to petri dishes for more beetle larvae culture. Beetle culture continued for several months to produce enough beetle numbers to be introduced to *Rumex crispus* plants.

Rumex crispus seedlings introduction to the beetle

Experiment were designed in completely randomized design with seven replicates and was carried out in the glasshouse with temperature ranging from 14 to 22 C. Adults beetles of *Gastrophysa virdula* were introduced to *Rumex crispus* plant populations at four levels; 0 Beetle as a control treatment or 1 Beetle or 2 Beetles or 3 beetles. Beetles were moved from containers and introduced to seedlings. Straightaway *Rumex* seedling spots were covered with a plan plastic pages and tightened with aelastic band to keep beetles inside the covered pots. Pots were placed randomly in an open plastic trays in the glasshouse. As normal watering was stopped as plants were covered by plastic pages, sponge layers were placed below the pots in the trays and kept always moistened with water to allow enough water available for *Rumex* crispus roots. Beetle grazing on *Rumex crispus* seedlings were allowed for 72 hours.

Plastic covers were then removed and beetles were returned back to containers. *Rumex* crispus seedlings residual and control pots were harvested. Dry weight and shoot numbers/pot were measured. Pots were taken out of trays and watered regularly to monitor their reaction after beetle grazing. Pots were left for three months with enough light and watering. The same procedure repeate deach time at the four leaf area of *Rumex crispus* stages under investigation (stage 1:1.22 cm2/plant, stage2:4.45 cm2/plant, stage3:11.56cm2/plant, stage: 71.52cm2).

RESULTS

Rumex crispus growth at the four seedling stages measured by leaf area/plant (pot)

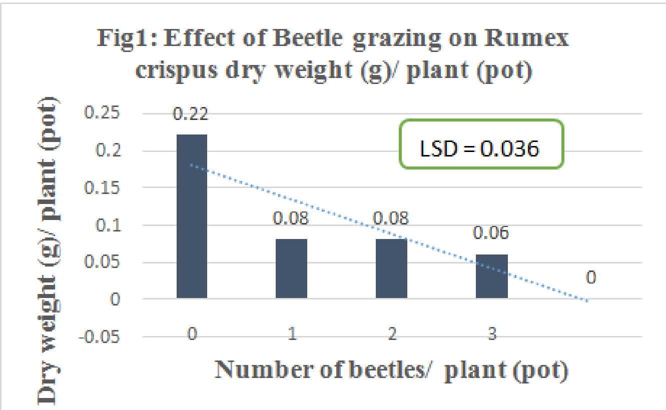
Table 1 below represents average dry weight (g/plant (pot)) and shoots number/plant (pot) for *Rumex crispus* at the four growth stages after beetle invasion including control treatment (0 beetle/plant (pot). Seedlings growth stage was measured by leaf area/plant (pot) at the time of introduction to beetle. The first, second and third growth stages of *Rumex crispus* did not differ significantly from each other in dry weight (g)/plant (pot) and shoots number/plant (pot). This is due to limited time between them (3 weeks intervals). However at stage four, *Rumex crispus* plants growth measurements differ significantly ($P \leq 0.01$) from previous stages as *Rumex crispus* seedlings have been left to grow seven weeks extra from third stage which is reflected in their growth measurements.

Table 1. ***Rumex crispus*** **dry weight (g/pot) and shoots number/ plant (pot) at the four growth stages**

Growth stage measured by average of leaf area/plant	Average of dry weight (g)/plant (pot)	Average of shoots number/plant (pot)
Stage 1 (average leaf area/plant:1.22 cm2)	0.0479	2.71
Stage 2 (average leaf area/plant:4.45 cm2)	0.0668	2.68
Stage 3(average leaf area/plant:11.56 cm2)	0.0775	2.79
Stage 4(average leaf area/plant:71.52 cm2)	0.2614	4.18
LSD at 5 %	0.0367	0.763

Effect of Beetle (*Gastrophysa virdula*) grazing on *Rumex crispus* dry weight

Beetle grazing resulted in a great reduction in *Rumex crispus* dry weight (g)/plant (pot). Statistical analysis showed significant reduction in the average of *Rumex crispus* dry weight (g)/plant (pot) at high level of of significance of (≤0.01) even in the present of only one beetle/plant (pot). The presence of two beetle did not increase the effect at all. This can be attributed to the competition between the beetle themselves or to the beetles conditions under plastic bags. It can be also attributed to the fact that some beetles completed their life cycle and died soon after their introduction to the weed. The increase tothree beetle increased slightly the reduction in the average of dry weight/plant (pot) although it was not significant and dramatic (Fig 1).



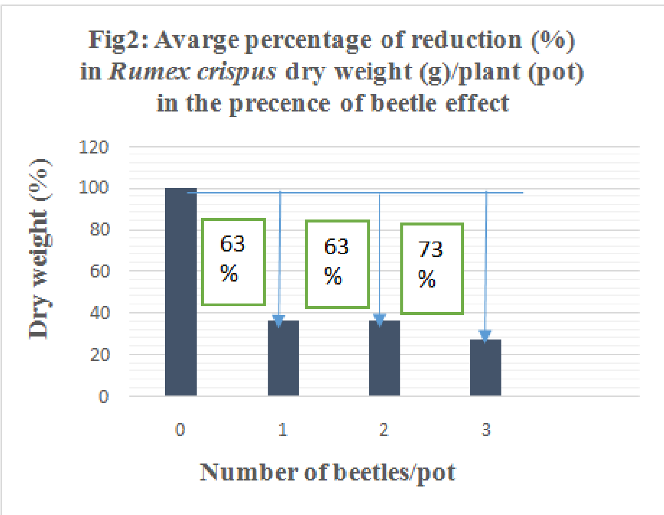
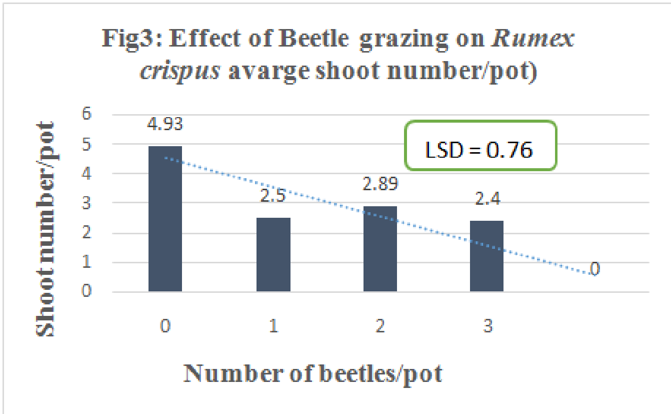
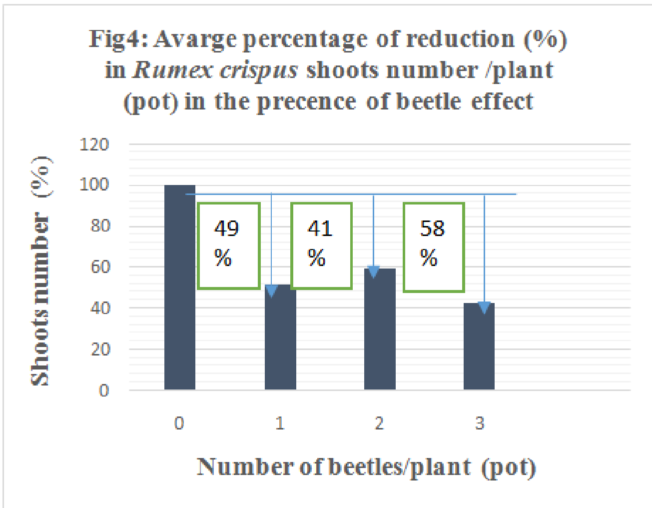


Figure 2 illustrates the percentage of reduction in the average of dry weight (g)/plant (pot) of *Rumex crispus* as a result of one or two or three beetles (*Gastrophysa virdula*) grazing. The present of one beetle reduced the average of dry weight (g)/plant (pot) of the four growth stages by 63%. The increase in number of beetles was not of significant effect confirming high ability of a single beetle (*Gostrophysa virdula*) to create great damageon one seedling of the weed.

Effects of Beetle (Gastrophysa virdula) grazing on Rumex crispus shoot numbers

Average of shoots number/plant (pot) of the different growth stages of *Rumex crispus* was also significantly reduced at high level of significance (≤0.01) as a result of one beetle (*Gastrophysa virdula*) grazing.





The increase in number of beetles/plant (pot) to two was of less effect although by none significant change. This can be attributed to the competition between the beetle themselves or to the beetle conditions under the plastic bags. It can be also attributed to the fact that some beetles completed their life cycle and died soon after their introduction to the weed. Three beetles grazing created more effect on the average of *Rumex crispus* dry weight (g)/plant (pot) but was not again of significant effect confirming great effect of one beetle on one seedling shoots number.

Figure 4 below illustrates the percentage of reduction in *Rumex crispus* shoot numbers /plant (pot) as a result of beetle grazing. The present of one beetle reduced shoot numbers/plant (pot) by 49%. The present of two beetles was of lower effect. Three beetlesgrazing reduced the number of shoots/plant (pot)of *Rumex crispus*by 58% although it was not considered to be a significant effect. Again one beetle was enough to create great damage on *Rumex crispus* seedlings.

Effect of the increase in beetles number/plant (pot) on *Rumex crispus* dry weight at different growth stages

($P \leq 0.01$) by the grazing of one beetle at all growth stages. This effect continued sometimes with the increase of beetle numbers/plant (pot) at the four growth stages. However, grazing effect of beetle was not significant with the increase in beetle number/plant (pot) in all examined four stages of *R. crispus* growth confirming the efficiency of one beetle to create considerable effect on one *Rumex crispus* seedling at all four growth stages. As mentioned before, the present of two beetles or three beetles was not of significant from one beetle and sometimes of less effect in some stages although by none significant change. This can be attributed to the competition between the beetle themselves or to the beetles conditions under plastic bags. It may be also attributed to the fact that some beetles completed their life cycle and died soon after their introduction to the weed. Figure 3 above illustrates the percentage of reduction in the average of dry weight (g)/plant (pot) of *Rumex crispus* as a result of one or two or three beetles

Table 2. Effect of the increase in beetle numbers on *Rumex crispus* average of dry weight (g)/plant (pot) at different growth stages

	Number of beetles/plant (pot)			
Growth stage measured by average of leaf area/plant	Control (0 beetle)	1 beetle	2 beetles	3 beetles
Stage 1 (average leaf area/plant:1.22 cm2)	00.070	0.0429	0.0371	0.0414
Stage 2 (average leaf area/plant:4.45 cm2)	0.1629	0.0229	0.0514	0.0300
Stage 3(average leaf area/plant:11.56 cm2)	0.2343	0.0211	0.0429	0.0100
Stage 4(average leaf area/plant:71.52 cm2)	0.4286	0.2329	0.2214	0.1629
LSD at 5 %	0.0735			

Table 3. Percentage (%) of reduction in *Rumex crispus* average dry weight (g)/plant (pot) at different growth stages as result of the increase in beetle numbers/plant (pot)

	Number of beetles/plant (pot)			
Rumex crispus growth stage measured by average of leaf area/plant	1 beetle	2 beetles	3 beetles	Mean
Stage 1 (average leaf area/plant:1.22 cm2)	39%	47%	41%	42.3%
Stage 2 (average leaf area/plant:4.45 cm2)	86%	69%	81%	78.6%
Stage 3(average leaf area/plant:11.56 cm2)	91%	82%	96%	89.7%
Stage 4(average leaf area/plant:71.52 cm2)	46%	49%	62%	52.3%

Table 4. Effect of the increase in beetles number/plant (pot) on *Rumex crispus* averageshoots number/plant (pot) at the different growth stages

	Number of beetles/plant (pot)			
Rumex crispus growth stage (measured by total leaf area/plant (pot))	Control (0 beetle)	1 beetle	2 beetles	3 beetles
Stage 1 (average leaf area/plant:1.22 cm2)	3.71	2.57	2.29	2.29
Stage 2 (average leaf area/plant:4.45 cm2)	4.71	1.29	3.00	1.71
Stage 3(average leaf area/plant:11.56 cm2)	5.71	1.71	2.71	1.00
Stage 4(average leaf area/plant:71.52 cm2)	5.57	4.43	3.57	3.14
LSD at 5 %	1.52			

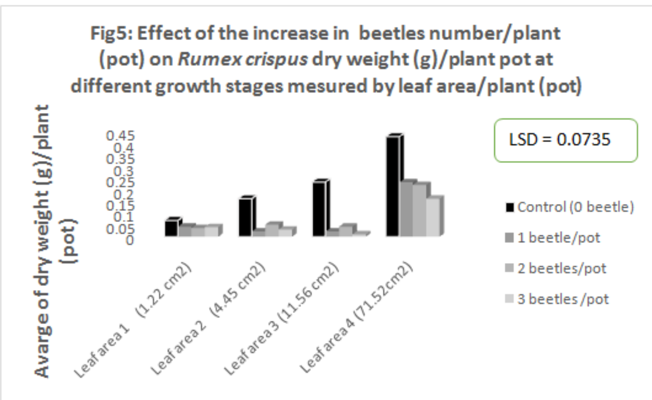


Table 2 and Figure 5 above showed that dry weight (g)/plant (pot) of *Rumex crispus* have been affected significantly

(*Gastrophysa virdula*) grazing at different growth stages of *Rumex crispus*. The present of one beetle reduced significantly the average of dry weight (g)/plant (pot) in the four growth stages by varying percentage. The increase in number of beetles was not of significant effect confirming high ability of a single beetle (*Gostrophysa virdula*) to create great damage on one seedling of the weed.

Effect of the increase in beetles/plant (pot) on *Rumex crispus* shoots number at different growth stages (measured by total leaf area/plant (pot))

Table 3 and Figure 6 showed that shoots number/plant (pot) of *Rumex crispus* have been affected significantly ($P \leq 0.01$) by the grazing of one beetle at all growth stages. This effect continued with the increase of beetle numbers/plant (pot). However, the increase in beetles number/plant (pot) to two or three was not of significant effect from the effect of one beetle in all examined four stages of *Rumex crispus* growth confirming again the efficiency of one beetle to create considerable effect on one *Rumex crispus*seedling at all four examined growth stages.The effect of increase in number of beetles/plant (pot) continued sometimes with the increase of beetles number/plant (pot) at the four growth stages. However, grazing effect of the beetle was not significant with the increase in beetle number/plant (pot) in all examined four stages of *Rumex crispus* growth confirming the efficiency of one beetle to create considerable effect on one *Rumex crispus* seedling at all four growth stages. As mentioned before, the present of two beetles or three beetles was not of significant from one beetle or even of less effect in some stages although by none significant change. This can be attributed to the competition between the beetle themselves or to the beetles conditions under plastic bags. It may be also attributed to the fact that some beetles completed their life cycle and died soon after their introduction to the weed.

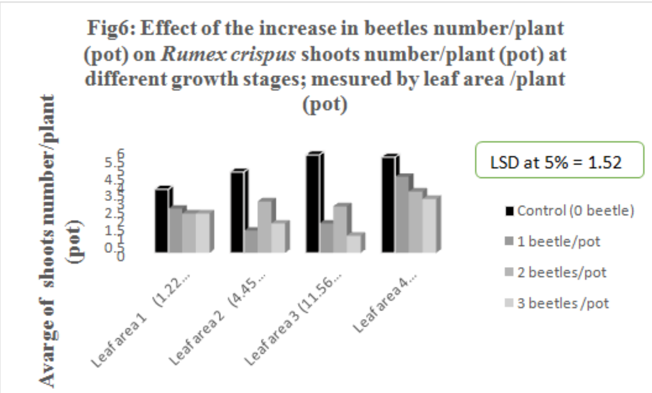


Fig6: Effect of the increase in beetles number/plant (pot) on *Rumex crispus* shoots number/plant (pot) at different growth stages; mesured by leaf area /plant (pot)

Correlation between growth measurements of *Rumex crispus*

Statistical analysis showed high correlation (0.77) between the measurements taken for *Rumex crispus* seedlings (dry weight (g)/plant (pot) and shoots number/plant (pot) at the four seedlings growth stages. This confirms similarity of the beetle effect on *Rumex crispus* growth at all examined growth stages. Moreover, it does show similar effect of beetle on the two measurements of *Rumex crispus* growth (dry weight (g)/plant (pot) and shoots number/plant (pot)). Also, it does show that the beetle grazing covers both leaves and tillers of *Rumex crispus*at similar amount and that the beetle grazing covers the whole dry matter of the weed *Rumex crispus*.

Re growth ability of *Rumex crispus* seedlings post beetle grazing

Table 3 above showed that after four months from beetle grazing, *Rumex crispus* seedlings were not able to re grow confirming to be dead especially at small seedling stages. At the biggest growth stage with leaf area of 71.52/plant (pot), some plants of *Rumex crispus* reemerged. This suggests that beetle significant effect is in early seedling stages. Combining beetle grazing with other control measure at old growth stages could give better result in suppressing the weed.

DISCUSSION

Measurements of *Rumex crispus* (dry weight (g)/plant (pot) and shoots number/plant (pot)) at first, second and third growth stages did not differ significantly reflecting limited difference in total leaf area/plant (pot).

Table 5. Percentage (%) of reduction in *Rumex crispus* shoots number/plant (pot) at different growth stages as result of the increase in beetle numbers/plant (pot)

	Number of beetles/plant (pot)			
Rumex crispus growth stage measured by average of leaf area/plant	1 beetle	2 beetles	3 beetles	Mean
Stage 1 (average leaf area/plant:1.22 cm2)	31%	38%	38%	35.7 %
Stage 2 (average leaf area/plant:4.45 cm2)	73%	37%	44%	53.3 %
Stage 3(average leaf area/plant:11.56 cm2)	71%	52%	82.5%	68.3 %
Stage 4(average leaf area/plant:71.52 cm2)	21.5	36%	43.7	33.7 %

Table 6. Number of emerging tillers of *Rumex crispus* four months after harvest

Rumex crispus Growth stage (Measured by leaf area/plant (pot)	Number of Beetle/plant (pot)			
	0 (control)	1	2	3
Stage 1 (average leaf area/plant:1.22 cm2)	2	0	0	0
Stage 2 (average leaf area/plant:4.45 cm2)	3	0	0	0
Stage 3(average leaf area/plant:11.56 cm2)	3	0	0	0
Stage 4(average leaf area/plant:71.52 cm2)	3	2	2	1

Table 5 above illustrates the percentage of reduction in the average of shoots number /plant (pot) of *Rumex crispus* as a result of one or two or three beetles (*Gastrophysa virdula*) grazing at different growth stages of *Rumex crispu*s. The present of one beetle reduced significantly the average of shoot numbers/plant (pot) in the four growth stages by varying percentage(21.5-73%). The increase in number of beetles was not of significant effect confirming high ability of a single beetle (*Gostrophysa virdula*) to create great damage on one seedling of the weed.

This is due to limited time between them (3 weeks intervals). However at stage four, *Rumex crispus* growth measurements differ significantly from previous stages as *Rumex crispus* seedlings have been left to grow seven weeks extra from third stage and have grown which is reflected in their growth measurements. Although *Rumex crispis* were at early growth seedlings stages, it provided considrable amount of leaves and green fresh matter expressing its capability as a troublesome weed. This is confirmed by its other growth characteristics including its high ability to germinate from seed, its persistent taproot system, high capacity of re growth after cutting and massive seed production (Pye, 2008). The Beetle *Gastrophy savirdula* grazing resulted in a great reduction in *Rumex crispus* avarge dry weight (g)/plant (pot) and shoot number/plant (pot). The present of one beetle reduced the average dry weight (g)/plant (pot) of the four growth stages of *Rumex crispus* by 63%. The presence of two beetles was not of further effect but the present of three beetles increased the reduction up to 73% although it was not statistically significant. This confirm the ability of one beetle to create 63% reduction in one seedling of the weed. The reduction was less and only up to 40% when mature plants of *Rumex obtusifolus* were infested heavily by the beetle (Person and Brooks, 1996).

The increase in number of beetles/plant (pot) was not of significant effect on the weed dry weight confirming high ability of a single beetle (*Gostrophy savirdula*) to create great and maximum damage on one seedling of the weed at the different examined seedlings stages of *Rumex crispus.* Average of shoot number/plant (pot) of the different growth stages of *Rumex crispus* was also greatly reduced as a result of one beetle (*Gastrophy savirdula*) grazing. The present of one beetle reduced shoot numbers/plant (pot) by 49% confirming one beetle ability to create maximum damage on one *Rumex crispus* seedlings. The increase in number of beetles/plant (pot) to two was not of effect however the increase to three reduced the number of shoot by 58% although it was not considered to be a significant effect confirming great effect of one beetle on one seedling shoots number.

This explains beetle grazing behavior toward *Rumex crispus* tillers confirming its ability to graze the weed tilers which could result in seedlings mortality. Looking at the effect of beetle on dry weight (g)/plant (pot) of *Rumex crispus* at each stage individually, the beetle grazing created significant damge on *Rumex crispus* dry weight (g)/plant (pot) in almost similar pattern in the four examined seedlings growth stages of *Rumex crispus*. This effect continued to increase with the increase of beetle numbers/plant (pot) at the four growth stages. However, increasing effect of beetle grazing was not significant with the increase in beetle number/plant (pot) in all examined four stages of *Rumex crispus* growth confirming again the efficiency of one beetle to create considerable effect on one *Rumex crispus* seedling at all four growth stages. Similarly, the effect beetle on *Rumex crispus* shoots number/plant (pot) at each stage individually as a result of beetle grazing was almost identical to the effect of the beetle on dry weight.

One beetle created significant damage on *Rumex crispus* shoots number/plant (pot) in almost similar pattern in the four examined seedlings growth stages. This effect continued to increase in the same pattern with the increase in beetle numbers/plant (pot) at the four growth stages although it was not significant. It is noticed that the increase in number of beetles/plant (pot) was not of significant effect on *Rumex crispus* growth in all examined growth stages. Moreover, in some stages, the present of two beetles was even of less effect than in the present of one beetle. This can be attributed to different factors related to the beetle feeding habit and its condition within grazing period. In this study, adults beetle were introduced to *Rumex crispus* for grazing.

It was reported that beetle during late larvae stage can express more rapacity in grazing of the weed (Martinkova and Honek, 2004). High correlation between the data measurements taken for *Rumex crispus* seedlings (dry weight (g)/plant (pot) and shoots number/plant (pot) at the four seedlings growth stages confirms similar grazing effect of the beetle on *Rumex crispus* growth at all examined growth stages which represent beetle rapacity even on *Rumex crispus* during seedlings growth stages. Moreover, it does show similar effect of beetle on the two measurements of *Rumex crispus* growth (dry weight (g)/plant (pot) and shoots number/plant (pot)) which reflect high suppression effect of beetle grazing on the weed.

Also, it does show that the beetle grazing covers both leaves and tillers of *Rumex crispus* at similar amount which could lead to high suppression and of the weed. *Rumex crispus* seedlings were not able to regrow after four months from beetle grazing confirming to be dead especially at small seedling stages. At the biggest growth stage with leaf area of 71.52/plant (pot), some plants of *Rumex crispus* reemerged. This suggests that beetle significant effect in early seedling stages could kill the weed. This result can't draw conclusion and it need to be investigated in a natural condition. Combining beetle grazing with other control measure at old growth stages could give better result in suppressing the weed (Hatcher *et al*, 1994). Although of promising result including the amount of reduction in weed growth as a result of beetle grazing which proves one beetle efficiency at different seedling stages this work need to be repeated in a larger scale and in a field condition to see the beetle behavior in the natural condition.

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