

## Research Paper

### **Exploring the Hormetic Response of *Pisum sativum* to Garden Cress (*Lepidium sativum*) Aqueous Extract: Implications for Natural Growth Enhancement**

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#### **Abstract**

Garden cress (*L. sativum*) and its allelopathic effects are the subject of increasing interest in sustainable agriculture research as a natural bio-stimulant. Due to the low concentrations of the extract, garden cress is suitable for use in ecological farming. This study explored how the root extract of garden cress (*L. sativum*) influences the growth and germination of *P. sativum* (pea) in a concentration-dependent manner. This study aimed to identify the optimal extract concentration to be used for the bio-stimulation of pea plants and for an effective delivery system to enhance crop species growth. Low extract concentrations (2.5% to 10%) increased the root and shoot length, as well as fresh weight, while high concentrations (40% to 80%) act as an inhibitor of growth. From a practical perspective, the extract at 2.5% concentration-maintained shoot length and promoted faster germination. For root fresh weight, an unexpected increase was observed, with an increase in shoot length at moderate concentrations (20% and 40%) which may be attributed to plant hormones induced by allelochemicals. Phytochemical analysis revealed several phenolic compounds, in which p-coumaric acid was the most abundant (5.93 ppm). Based on the dose-response curve for shoot fresh weight, it was found that an EC50 value of 25.4%, is an important indicator for future concentrations to be used. Responses show that low garden cress biomass concentrations should be applied for sustainable agriculture to avoid allelopathy's negative effects on crop species. In summary, our work also emphasizes the importance of low doses and why allelopathy can be exploited for crop improvement, as well as serving as a substrate as part of a closed nutrient cycle in sustainable agriculture. Further research is required to examine the longer-term effects of this plant-plant interaction on crop yield and ecosystem services.

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### 1. Introduction

Scientific understanding of how plants interact with each other in agroecosystems has a long history but is growing as researchers increasingly explore opportunities to use natural plant-to-plant interactions to improve the sustainability of agricultural practices (Ratnadass et al., 2011; Kremen & Miles, 2012). Plant-to-plant interactions include the hypothesized production of allelochemicals by certain plants to inhibit or alter the growth, development, and functioning of other plants (Hu et al., 2018). Hence research attention is focused on exploiting such chemical signals for improved crop performance and greater environmental sustainability. Using plants with high levels of phytochemicals like phenolics and flavonoids which also exhibit growth-promoting properties, can insinuate beneficial effects on crops. Garden cress (*L. sativum*) is an edible, fast-growing herb of interest here (Adera et al., 2022).

Studies have shown that plant-produced volatiles can have stimulatory or inhibitory effects on neighboring plants dependent on their concentration and the species of target plant (Paudel et al., 2020). Biphasic dose response, where concentrations of a compound stimulate responses to low doses yet inhibit at high doses, is known as hormesis (Calabrese et al., 2008). These hormetic effects have been demonstrated in many species of plants and can be used in the development of weeding and crop enhancement strategies (Dascaluic et al., 2020).

*P. sativum* (pea), an important leguminous crop, is one of the most inclusive models for testing phenotypic modulation of plant growth with plant extracts due to its worldwide agricultural significance (Akemo et al., 2000). As a legume, peas are useful because of their nitrogen-fixing capability through rhizobia, a symbiotic bacterium that can be a key component in increasing soil fertility and pH and can provide a valuable source of protein (Goyal et al., 2021). By modulating pea growth and development naturally, new approaches could be explored to increase yields and reduce the number of synthetic inputs needed (Kreplak et al., 2019).

The phenolic compounds identified in the phytochemical composition of garden cress such as p-coumaric acid, gallic acid, vanillic acid, and quercetin might play an important role in influencing the germination and growth of other plants and might also harm biological activities including antioxidant biological activities and growth (Mohamed et al., 2023). However, the influence of the exogenous application of extract on the growth and germination of pea plants such as root length, shoot length, germination time, germination indices, germination percentage, and chlorophyll fluorescence at different concentrations of garden cress extract has not been investigated fully (Gupta et al., 2021).

For any number of reasons, a clear grasp of the concentration-dependent effect of garden cress extract on pea development holds enormous promise. First, it might lead to ways to use low doses of the extract as a natural bio stimulant, to potentially enhance crop performance without chemical growth enhancers (Tufail, 2024). Second, mapping out the hormetic response curve should provide clues regarding fundamental mechanisms of plant-to-plant interactions that could inform our basic understanding of allelopathy and plant ecology (Islam and Kato-Noguchi et al., 2013).

Furthermore, when garden cress extract is tested on different growth characteristics of peas, such as root and shoot length, total biomass accumulation, and germination traits, more detailed information on how the extract influences pea growth will be available (Ajdanian et al., 2019). This allows us to assess how the extract acts as a growth booster and ensures that we understand whether there are conflicting effects on different aspects of plant growth, meaning trade-offs (Marinov-Serafimov et al., 2010).

This research postulates to explain the concentration-dependent effects of garden cress aqueous extract on the growth and germination of *P. sativum*. The aim is to determine the response of peas to garden cress extract and to see if this response is hormesis by examining a wide range of growth parameters at different concentrations of garden cress extract (Nazir et al., 2021). This research not only looks at the potential to utilize garden cress as a natural growth enhancer to peas but, perhaps more importantly, it contributes to the increasing number of studies on the topic of plant-plant interactions towards sustainable agriculture.

Additionally, this exploration of the biphasic effects of garden cress extract on pea growth is also aligned with the current concerns about environmentally friendly agricultural improvements (Abiola et al., 2021). The discovery of natural growth promoters derived from plants could become a basis for further research and utilization in developing eco-friendly strategies for resource-efficient crop enhancement (Shayen et al., 2023). The findings of this study could also open new pathways to organic farming and integrated pest management strategies, where the targeted usage of plant metabolites could provide a beneficial and more environmentally safe crop growth improvement (Jacoby et al., 2020).

Our study aims to offer an integrative assessment of the effect of garden cress aqueous extract on the pea growth and germination parameters in different levels of concentration to identify the potential for low-dose bio stimulation and the concentrations at which stimulatory effects transform into inhibitory ones.

## 2. Material and Methods

### 2.1. Experimental site

The research was conducted at Weed Science Laboratory, Department of Agronomy, University of Agriculture, Faisalabad. The study was carried out using CRD under experimental conditions using three replications. In a laboratory experiment, the summer vegetable garden cress with winter vegetable peas observed the allelopathic potential of peas against weeds. Collected dried samples like seed and wood were separated into two different parts and chopped into small lengths managed as about 2 cm. The chopped sample was soaked separately in the ratio of 1:80 in water and serial dilution was made in it then passed through cotton cloth to get water extracts of *L. sativum*. So diluted concentrations of an extract with the ratio of control 0, 25, 50, 10, 20, 40, and 80% maximum extract were used. This extract of *L. sativum* was applied to *P. sativum* in the experiment. For this experiment garden cress extract was applied to the peas in Petri plates of three replications, each containing 10 seeds of pea wrapped in these petri plates. This experiment contains seven treatments like,  $T_1$  = distilled water (control),  $T_2 = 2.5\%$ ,  $T_3 = 5\%$ ,  $T_4 = 10\%$ ,  $T_5 = 20\%$ ,  $T_6 = 40\%$ ,  $T_7 = 80\%$ .

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### 2.2. Observations

#### 2.2.1 Germination percentage (%)

The numbers of seeds grown were counted every day up to 12 days afterward the observation stopped. Seeds were considered sprouted when the shoot had attained 1-2 cm length. The following formula was used to count the germination percentage.

$$GP = \frac{\text{Germinated Seeds at final count}}{\text{Total Seeds}} \times 100$$

#### 2.2.2. Time to 50% germination (T50)

The time to 50% emergence (T50) was determined by using the formula of (Cool bear *et al.*, 1984) which is improved further by (Farooq *et al.*, 2005).

$$T_{50} = ti + \frac{\left(\frac{N}{2} - ni\right)(tj - ti)}{(nj - ni)}$$

Where N is the number of seeds that germinate, and nj and ni are the cumulative number of seeds that emerge at times tj and ti, where ni < N/2 < nj.

#### 2.2.3. Mean germination time (MGT)

Mean emergence time (MET) was calculated according to the equation of (Ellis and Reborts, 1981).

$$MGT = \frac{\sum Dn}{\sum n}$$

Where n is the number of seeds that emerged on Day, and D is the number of days counted from the beginning of germination.

#### 2.2.4. Germination index (GI)

The germination index was calculated by the formula of Association of Official Seed Analysis (AOSA, 1983).

$$GI = \frac{N_1}{D_1} + \dots + \frac{N_L}{D_L}$$

Where D<sub>1</sub> is the day to first count, N<sub>1</sub> is the no. of germinated seeds on the first day, N<sub>L</sub> is the no. of germinated seeds at the final count and D<sub>L</sub> are days to final count.

#### 2.2.5. Root length (mm)

All the seedlings of each replication were taken after 12 days, and the length of each root was measured by the scale in mm from the point where the root and shoot joined and separated the root from that joining point. The average root length was worked out.

#### 2.2.6. Shoot length (mm)

All the seedlings of each replication were taken after 12 days, and the length of each shoot was measured by the scale in mm from the point where the shoot and root joined together and separated the shoot from the joining point. Then average length of the shoot was calculated.

### **2.2.7. Fresh weight of shoot (mg)**

Shoots of all the seedlings were separated and the weights of each treatment in each replication were taken separately. The weight of each plant was calculated in mg by dividing the total weight by the total number of shoots.

### **2.2.8. Fresh weight of root (mg)**

The roots of all the sprouts were detached and the mass of each treatment in each replication was taken separately. The weight of each plant was determined.

### **2.2.9. Phenolic contents**

The phenolic contents given in (Table. 1) of this research were measured by HPLC (Gradient, Reverse phase made from Shimadzu Japan; detector SPD-10AV pump LC -10AT). 10g of Garden cress powder was taken in 90% methanol. Samples were kept in a beaker covered with aluminum foil for 8 days. After 8 days the material was dried and 5mg was taken out for phenolic analysis. In *L. sativum* seed extract there is Quercetin, Gallic acid, Vanillic acid, and P-conmeric acid were detected.

### **2.10. Statistical Analysis**

Data was examined using Fisher's Analysis of Variance ANOVA. The difference among treatments was separated using the Least Significant Difference Test (LSD) (Steel *et al.*, 1996).

### **2.11. Measurement of dose-response relationship**

The dose-response relationship of the allelopathic compound on weed growth was modeled mathematically using an automated procedure based on curve fitting. For this purpose, a software called Dr-Fit (Di Veroli, 2015) was employed. Dr-Fit is part of a growing class of robust methods for modeling complex dose-response curves which can be monophasic (i.e., single piecewise linear) or multiphasic (i.e., with one or more transitions between linearity and curvature). Experimental data on plant growth are fitted to candidate models representing monophasic and/or multiphasic formulations of the relationship between exposure to a phytotoxic compound and plant growth. The fitting procedure is based on optimization by maximum likelihood and controlled by a BIC-based model selection procedure. The optimization is performed using a trust-region-reflective non-linear optimization algorithm. Fitting parameters are weighted by the standard deviation of replicate measurements, i.e., a proper way of performing the correct treatment of heteroscedasticity in dose-response data. The baseline response is a free parameter with a fixed value (set to unity), as it represents the minimum response (in control conditions). The best BIC score turned out to be a biphasic function, with an initial stimulatory phase characterized by a positive interaction between the two species at low allelochemical concentrations, and a subsequent inhibition phase at high doses.

This hormetic response was formalized using a two-process model that includes terms from stimulatory and inhibitory Hill equations:

$$E(C) = [1 + (E^{\infty 1} - 1)/(1 + ((Ec50\_1/c)^{H1})] \times [1 + (E^{\infty 2} - 1)/(1 + (c/(EC50\_2)^{H2}))]$$

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Where  $E_c$  is the effect at concentration  $C$ ,  $E_{\infty 1}$  and  $E_{\infty 2}$  are the maximal effects of the stimulatory and inhibitory process respectively;  $EC50_1$  and  $EC50_2$  are half-maximal effective concentration; and  $n_1$  and  $n_2$  are the Hill slopes of each process.

That mathematical descriptor in turn provides an analytical scheme for evaluating the highly nonlinear dose-dependent action of the allelopathic chemical on vegetable growth, with the additional benefit that this can be interpolated, extrapolated, and relevant toxicological parameters derived.

In this case, we optimized the fitting parameters with the trust-region reflective algorithm; we weighed the fitting parameters by their standard deviations; and accounted for the baseline as a fitted parameter. This quantitative framework can be utilized to analyze the often-complex dose-related allelopathic effects on vegetable growth.

### 3. Results

The results of the experiment show that the effect of the aqueous extract of garden cress (*L. sativum*) on the growth and germination characteristics of *P. sativum* (pea) is quite complex. Firstly, it is evident from the data that the growth of plants in the first 5 days is stimulated by the very low concentrations of the extract, while higher concentrations reduce growth. Overall, analysis of root and shoot length measurements showed a downward trend of growth with an increase in the concentration of extracts.  $T_1$  or control treatment with 0 % extract had a higher root length at 37.0 mm compared to all other treatments which were significantly different. This was attributed to the fact that the sample with 2.5 % extracts ( $T_2$ ) had the same shoot length as the control (37.0 mm), but the root length was slightly reduced (29.3 mm), probably due to a stimulating effect on shoot growth at such a low concentration. The data on the fresh weight of roots shows an interesting pattern. In most of the treatments, the fresh weight of the roots did not differ significantly from the control (63.3 mg), while 20% and 40% extract led to an increase of the root fresh weight to 68.0 mg and 69.0 mg correspondingly. It is possible that the plants underwent growth-promoting effects at moderate concentrations of the extract. Germination parameters further explain the mechanism of the extract action on pea germination and growth. The mean emergence time (MET) ranged from 6.6 to 8.0 days across treatments. The MET in  $T_2$  (2.5% extract) resulted in the fastest emergence. Also, the Germination percentage remained high (81.3% to 89.3%), across all treatments, with no statistical differences observed. However, the germination index, which is an integration of the speed and completeness of germination, ranged from 5.8 to 7.0 days, with the highest value in  $T_4$  (10% extract). This implies that the germination process might have been stimulated by the 10% extract concentration, thus the highest overall germination performance was observed. The time to 50% germination data shows a nonlinear response to increasing extract concentrations. The lowest concentration of the extract (2.5%,  $T_2$ ) resulted in the shortest time to 50% germination (4.3 days) and the highest concentration (80%,  $T_7$ ) resulted in the longest time to 50% germination (6.2 days), therefore 2.5% concentration of garden cress extract might speed up the pea germination process.

Another evidence that the garden cress extract exerts a complex action on shoot fresh weight of Pea is the result of a fitted biphasic dose-response curve (1 inhibition + 1 stimulation), as shown in the figure below. According to the result of (Table. 3) the garden cress extract

exhibits an initial stimulatory effect at lower concentrations, followed by an inhibitory effect at higher concentrations. The EC50 value that indicates the concentration eliciting 50% of the maximum effect was calculated to be 25.4%. Analysis (phytochemical) of the aqueous extract of garden cress showed the presence of several phenolic compounds. P-coumaric acid (5.93 ppm) was the most abundant followed by gallic acid (4.82 ppm), vanillic acid (1.94 ppm), and quercetin (0.52 ppm). These compounds are known for their bioactivities and may be responsible for the effects observed on pea growth and germination. The mechanisms of these effects could be related to physiological and biochemical pathways.

In conclusion, our results showed that the aqueous extract of *L. sativum* acts on growing and germinating *P. sativum* in a concentration-dependent manner. Lower concentrations, especially in the range of 2.5% - 10%, may have beneficial effects on some parameters of growth and germination. At the same time, the highest concentrations may certainly have inhibitory effects. This study is a starting point for the investigation of the properties of garden cress extract useful for developing a bio stimulant with optimization.

**Table. 1:** Phytotoxic effect of garden cress aqueous extract on shoot length, root length, root fresh weight, shoot fresh weight, time to 50% germination, germination index, mean germination, and germination percentage of pea.

Treatments	Root Length (mm)	Shoot Length (mm)	Root fresh weight (mg)	Shoot fresh weight (mg)	Mean Emergence Time (MET)	Germination %	Germination index	50% Emergence
T <sub>1</sub> =0% (Control)	37.0a	37.0a	63.3ab	66.6b	7.5	88.3a	5.4bc	5.5b
T <sub>2</sub> =2.5%	29.3b	37.0a	60.7ab	76.6a	6.6	84.3a	5.9bc	4.3d
T <sub>3</sub> = 5%	26.6b	29.3b	59.9a	70.0b	7.5	85.3b	6.3ab	5.3bc
T <sub>4</sub> = 10%	23.5cd	26.6b	60.0ab	60.0c	7.4	89.3b	7.0a	4.6cd
T <sub>5</sub> = 20%	20.2de	23.5cd	68.0bc	53.3d	7.4	87.0a	4.9c	5.7ab
T <sub>6</sub> = 40%	16.4e	20.2de	69.0d	46.1e	8.0	82.3b	5.3bc	5.7ab
T <sub>7</sub> = 80%	17.0e	16.4e	51.3cd	36.6f	7.9	81.3a	5.1c	6.2a

Mean values followed by different letters are statistically different from each other at a 5% level of probability.

**Table. 2:** Phenolic compounds detected in aquatic extract of garden cress

Garden Cress		
Sr. No.	Phenolic compounds	Concentration in ppm
1	Quercetin	0.52
2	Gallic acid	4.82
3	vanillic acid	1.94
4	P-conmeric acid	5.93

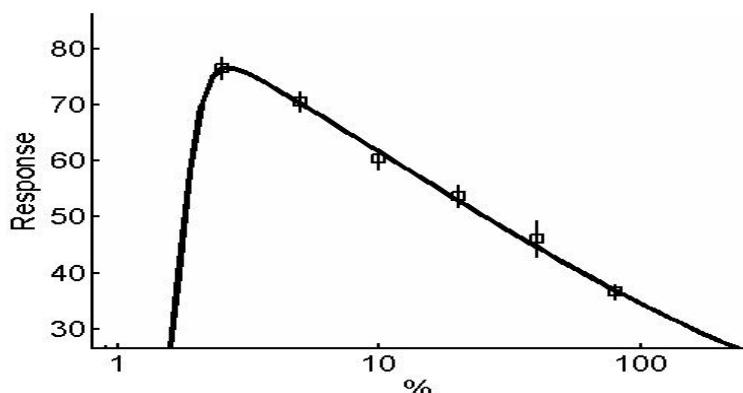
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### 3.1. Potential hermetic dose range of Garden cress for shoot fresh weight of *P. sativum*

The effect of the garden cress aqueous extract on the shoot fresh weight of *P. sativum* was investigated using dose-response curve data (Fig. 1). The relationship between the extract concentration and shoot fresh weight in *P. sativum* showed a biphasic tendency for shoot fresh weight, where there was a stimulatory effect with increasing concentration. Increasing concentrations led to an inhibitory effect on the shoot's fresh weight at a certain point and above. When tested against different concentrations, a biphasic model can be considered as a good description of hormetic response while the monophase model appears to represent a poorer description of the experimental data. The shoot fresh weight of *P. sativum* was slightly stimulated at low extract concentration (1-5%) with mean values slightly higher than in the control treatment. However, above 5% there was a pronounced inhibitory effect with the length rapidly falling in the interval between 10% and 50% of extract concentration.

The EC50 was 25.4 percent, or the concentration of the extract that reduced shoot fresh weight to half of the control. This is a quantifiable value that allows us to describe the inhibitory activity of the extract against the shoot fresh weight of *P. sativum*. The steepness of the curve around the EC50 indicates that a rather narrow range of concentrations results in the transition from minimum to maximum inhibition. At the highest dose (100 percent), inhibition was not total, since the shoot fresh weight was reduced to around 35 percent of the control. The dose-response curve appeared to be approaching a plateau at concentrations above 50 percent and the maximum level of inhibition was likely reached within the tested range.

In this sense, the observed hormesis (stimulation at low- vs inhibition at high dose) agrees with the evidence available in the allelopathy and plant ecology literature that supports the biphasic nature of the effect. Once again, the results indicate the complexity of plant-plant interactions, but more importantly, they suggest that the non-monotonic response should be considered when studying phytotoxicity. Garden cress proved to be a strong allelopathic species against *P. sativum*, a species that has been a major agricultural pest for centuries as an emerging weed among cereal crops. The estimated EC50 and the dose-response relationship would help to design applications for the management. Most importantly, any eventual implementation would have to take into consideration the dose used.



**Figure 1:** Dose-Response curve of garden cress aqueous extract on shoot fresh weight of *Phalaris minor* fitted through Biphasic (1 inhib. + 1 stim.) {x-axis: concentration (%) of aqueous extract of garden cress, Y axis: shoot fresh weight (mg)}.

**Table. 3:** Parameters of the model of dose-response Biphasic (1 inhib. + 1 stim.)

Parameters	Biphasic (1 inhib. + 1 stim.)
IC50_1	0
H_1	0
Emax_1	0
IC50_2	1.71991
H_2	9.92556
Emax_2	120.78
IC50_3	10.9798
H_3	0.41454
Emax_3	4.83E-12
Scaling	1
Chi2	27.9668
GOF	0.26144
aic	67.9336
bic	235.934
EC50	25.40%

#### 4. Discussion

Stimulatory and inhibitory effects, a complex but predictable biphasic effect stimulate growth and higher recent studies of plant bio stimulants and are expected to play an increasing role in sustainable agriculture in the years to come.

The apparent stimulation occurs in certain growth parameters at (2.5% to 10%) extract. For example, shoot length and germination were supported with garden cress extract at these lowest concentration levels. Our results agree with the previous findings indicating that low doses of allelochemicals (Wang et al., 2022).

The increased root fresh weight observed at moderate extract concentrations (20% and 40%) is intriguing and warrants further investigation. Most reflections of hormetic stress responses in which plants invest more in root growth, response to the stress of the nanomaterials, and other extract constituents (Al-Khayri et al., 2023). Such responses have been documented for several other species grown under both small and large concentrations of mild abiotic or biotic stressors and usually lead to an increase in plant performance (Pandey et al., 2017).

The phytochemical analysis showing the presence of phenolic compounds in the garden cress extract sheds light on the mechanisms behind the observed effects: p-coumaric acid, in the highest amount, has been previously described as causing growth promotion or inhibition in plants, depending on its amount (Bhaigyabati et al., 2017). The interactions between these phenolic compounds most likely contribute to the complex dose-response relationship observed in this study (Pandey et al., 2017).

The estimated EC50 for shoot fresh weight is 25.4%, effects on *P. sativum*, clearly indicate a threshold for future experiments and possible applications of optimized extracts for growth promotion, while avoiding inhibitions.

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In addition to the previous examples, these data add further support to the promising role of plant-derived compounds in natural bio stimulants in this kind of agriculture to determine if such treatments may affect crop yield and quality (Martinez-Lorente et al., 2024). This study underlines the complexity of plant-plant communications mediated by the allelochemicals and validates the use of garden cress extract as a new natural tool for pea improvement. The plants' hormesis reveals the importance of the dose for the use of natural molecules to obtain the desired responses to design ecological crops.

### **Conclusion**

In conclusion, I can say that the shoot and root elongation of *P. sativum* were inhibited by garden cress aqueous extract. Also, there is a decrease in fresh weight and germination of *P. sativum* at garden cress aqueous extract. And based on this finding garden cress aqueous extract can be used as herbicide. If the garden cress has potential as an herbicide further study is required about extract application areas in agriculture and horticulture such as hindering unwanted plant species development.

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### **Authors' contributions**

B.K. contributed data analysis and experimental work, I.A. wrote the manuscript, and R.M., M.A.N. designed the experiment and critically revised it.

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### **Declarations**

### **Competing interests**

The authors declare no conflict of interest.

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## Hormetic Response of *Pisum sativum* to Garden Cress (*Lepidium sativum*)

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