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Original Article

Allelopathic Effects of Three Plant Species on the Germination and Growth of Agricultural Crops: Isolation and Spectroscopic Characterization of Active Allelochemicals

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Abstract
Allelopathy plays a crucial ecological role in regulating plant interactions and maintaining ecosystem balance through the release of secondary metabolites known as allelochemicals. The present study investigated the allelopathic effects of three plant species — *Taraxacum officinale*, *Malva parviflora*, and *Solanum nigrum* — on the germination and early growth of three crops (*Triticum aestivum*, *Vicia faba*, and *Lolium perenne*). Aqueous and organic extracts from different plant parts were prepared and evaluated under controlled laboratory conditions to determine their inhibitory potential on seed germination, radicle elongation, and seedling biomass. The results revealed that all three donor plant extracts exhibited significant inhibitory effects, with *S. nigrum* extracts showing the strongest suppression on seed germination and root elongation, particularly in *L. perenne*. The degree of inhibition increased with extract concentration and varied according to both the donor and target plant species. Phytochemical screening and spectroscopic analyses (UV–Vis, FTIR, and NMR) identified several bioactive allelochemicals, including phenolics, flavonoids, and alkaloids, which are likely responsible for the observed allelopathic activity. These findings suggest that the tested plant species possess strong allelopathic potential, highlighting their ecological role and possible use as sources of natural bioherbicides. The study contributes to understanding plant–plant interactions in agroecosystems and supports the development of environmentally friendly weed management strategies based on allelochemical compounds.
Keywords. Allelopathy, Allelochemicals, *Taraxacum Officinale, Malva Parviflora, Solanum Nigrum.*

Introduction

Allelopathy is a critical ecological mechanism through which plants influence the germination, growth, and development of neighboring species by releasing biologically active secondary metabolites known as allelochemicals [1]. These natural compounds, secreted through volatilization, root exudation, or decomposition of plant residues, can either inhibit or stimulate the physiological processes of target plants [2]. In agroecosystems, allelopathy plays a significant role in shaping plant community structure and regulating crop–weed dynamics, offering a sustainable basis for developing natural herbicides [3].

During the past two decades, the ecological and agronomic implications of allelopathic interactions have attracted wide attention. Numerous studies have demonstrated that allelochemicals such as phenolic acids, flavonoids, terpenoids, and alkaloids exhibit strong phytotoxic properties that suppress seed germination and seedling growth of competing plants [4-5]. For instance, extracts of *Sorghum bicolor* and *Helianthus annuus* have been shown to inhibit the germination of several weed species [6], while Eucalyptus globulus leaf litter releases volatile compounds that reduce the growth of understory vegetation [7]. Among the broad diversity of allelopathic plants, *Taraxacum officinale*, *Malva parviflora*, and *Solanum nigrum* are particularly noteworthy because of their wide distribution, rich phytochemical composition, and strong ecological adaptability. *T. officinale* (common dandelion) produces sesquiterpene lactones and phenolic acids that interfere with germination and nutrient uptake in neighboring plants [8]. *M. parviflora* (small mallow) contains flavonoids and malvalic acid derivatives known for antimicrobial and phytotoxic properties [9]. *S. nigrum* (black nightshade) is rich in steroidal alkaloids and glycosides that have been associated with inhibitory effects on seedling growth [10].

Despite these insights, comparative assessments of the allelopathic potential of these three species—particularly in relation to economically important crops such as wheat (*Triticum aestivum*), faba bean (*Vicia faba*), and ryegrass (*Lolium perenne*)—remain limited. Spectroscopic identification of allelochemicals using advanced analytical tools such as ultraviolet–visible (UV–Vis), Fourier transform infrared (FTIR), and nuclear magnetic resonance (NMR) spectroscopy has improved our understanding of plant-derived bioactive compounds [11]. These methods enable precise detection of functional groups and structural elucidation of

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complex secondary metabolites responsible for allelopathic effects. Integrating these techniques into allelopathy research enhances the ability to correlate observed bioassays with chemical composition, providing a mechanistic basis for allelopathic activity [5]. This study, therefore, aims to evaluate the allelopathic effects of *T. officinale*, *M. parviflora*, and *S. nigrum* on the germination and early growth of selected crops and to isolate and characterize the major allelochemicals responsible for these effects using spectroscopic methods. The research will contribute to the ecological understanding of plant–plant chemical interactions and support the development of environmentally friendly weed-management strategies based on natural allelochemicals.

Materials and Methods

This section describes in detail the experimental design, materials, and analytical procedures used to evaluate the allelopathic effects of three selected plant species (*Taraxacum officinale*, *Malva parviflora*, and *Solanum nigrum*) on the germination and growth of crops. It also outlines the methods employed for the isolation and spectroscopic characterization of active allelochemicals.

Study Area and Plant Material Collection

The study was conducted in the El-Gabel Al-Khader region, located in northeastern Libya, characterized by a Mediterranean climate with mild, wet winters and hot, dry summers. Fresh plant materials of *Taraxacum officinale*, *Malva parviflora*, and *Solanum nigrum* were collected from natural habitats during the flowering stage (Figure 1). Plant identification was confirmed by the Department of Botany, Omar Al-Mukhtar University. The aerial parts were washed, shade-dried at room temperature, and ground into fine powder for further extraction [1-12].

Figure 1. Study plants (Taraxacum officinale, Malva parviflora, and Solanum nigrum)

Preparation of Aqueous and Organic Extracts

Aqueous extracts were prepared by soaking 100 g of powdered plant material in 1000 mL of distilled water for 24 hours at room temperature with occasional shaking. The extracts were filtered using Whatman No. 1 filter paper and concentrated under reduced pressure using a rotary evaporator at 40°C. Organic extracts were prepared using methanol and chloroform solvents with the same ratio and method. All extracts were stored at 4°C until use.

Phytochemical Screening

Preliminary phytochemical screening of the crude extracts was performed following standard methods to detect major secondary metabolites, including alkaloids, flavonoids, phenolics, tannins, saponins, and terpenoids [13-4].

Germination and Growth Bioassay

Bioassays were conducted to assess allelopathic effects on wheat (*Triticum aestivum*), faba bean (*Vicia faba*), and ryegrass (*Lolium multiflorum*). Ten seeds of each test crop were placed on filter paper in Petri dishes and moistened with 5 mL of each plant extract at concentrations of 25%, 50%, 75%, and 100%. Controls received distilled water only. Petri dishes were incubated at 25°C under a 12-hour light/dark cycle. Germination percentage, root length, and shoot length were measured after 7 days. Each treatment was replicated three times [14].

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Isolation and Purification of Active Allelochemicals
The crude extracts exhibiting the highest allelopathic activity were subjected to column chromatography using silica gel as the stationary phase and eluted with increasing polarity solvents (chloroform: methanol). Fractions were monitored using thin-layer chromatography (TLC) and visualized under UV light (254 and 366 nm). The fractions showing similar Rf values were pooled and concentrated.

Spectroscopic Characterization (UV–Vis, IR, NMR)
The purified allelochemicals were characterized using spectroscopic techniques. UV–Vis spectra were recorded on a Shimadzu UV-1800 spectrophotometer within the 200–800 nm range. FTIR spectra were obtained using a PerkinElmer Spectrum Two spectrometer with KBr pellets to identify functional groups. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 400 MHz spectrometer using deuterated solvents. Peaks were compared with published spectral data for compound identification [15].

Sample Preparation
Plant materials (*Taraxacum officinale, Malva parviflora,* and *Solanum nigrum*) were air-dried at room temperature and ground into a fine powder. Aqueous extracts were prepared by soaking the powdered plant material in distilled water (w/v) for 24 h at room temperature with intermittent shaking, followed by filtration through Whatman No. 1 filter paper. Organic extracts were obtained using sequential solvent extraction with ethyl acetate, dichloromethane, or chloroform depending on the plant species. The organic phases were concentrated under reduced pressure using a rotary evaporator and stored at 4 °C until analysis.

Purified fractions obtained from column chromatography and HPLC were dissolved in spectroscopic-grade methanol. All samples were filtered through 0.45 µm membrane filters prior to spectroscopic measurements.

FTIR Analysis
Fourier Transform Infrared (FTIR) spectroscopy was performed for functional group characterization. Spectra were recorded using an FTIR spectrometer in the range of 4000–400 cm^{-1}. Samples were prepared using the KBr pellet method. Measurements were carried out at a resolution of 4 cm^{-1} with multiple scans averaged for each spectrum.

UV–Visible Spectrophotometric Analysis
UV–Vis spectra were recorded by dissolving purified compounds in methanol and scanning between 200 and 400 nm using methanol as a blank reference. Absorption maxima (λmax) were recorded and compared with reported data for phenolic compounds.

Mass Spectrometric Analysis
Mass spectrometric analysis was conducted to determine molecular weights and fragmentation patterns. Samples dissolved in methanol were analyzed under electron ionization conditions, and spectra were recorded over the appropriate m/z range.

Statistical Analysis
Data obtained from germination and growth experiments were analyzed using one-way ANOVA (SPSS v25). Means were separated using Duncan's Multiple Range Test at $p \leq 0.05$ to determine significant differences among treatments [16].

Results and Discussion

Germination and Seedling Growth Bioassay
The allelopathic effects of *Taraxacum officinale*, *Malva parviflora*, and *Solanum* nigrum extracts on the germination and seedling growth of wheat (*Triticum aestivum*), faba bean (*Vicia faba*), and ryegrass (*Lolium multiflorum*) were evaluated under controlled conditions. Results showed a significant inhibition of germination and root elongation in all test crops, particularly at higher extract concentrations (75% and 100%). Among the three plants, *Solanum nigrum* exhibited the strongest inhibitory effect, reducing wheat germination by 68% compared to the control (Figure 2), followed by *Malva parviflora* and *Taraxacum officinale* (Table 1). This suppression is attributed to the presence of *phenolics*, alkaloids, and terpenoids that interfere with hormonal balance, enzyme activity, and nutrient uptake in germinating seeds [4-17]. Similar allelopathic inhibition by Solanum species was reported by [18], confirming that secondary metabolites such as solanine and solasodine disrupt cellular respiration and membrane integrity (Figures 3,4).

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Table 1. Germination percentage and seedling growth at different extract concentrations]

Conc. (gm. D.W/ 100 ml)	Germination		Shoot length (cm)		Root length (cm)	
	%of germinated seeds	R%	Mean	R%	Mean	R%
Control	86.67	0.00	2.90	0.00	4.17	0.00
2.5	73.33	15.38	1.93	33.33	3.70	11.20
5	66.67	23.08	1.93	33.33	2.00	52.00
10	53.33	38.46	0.97	66.67	2.17	48.00
15	53.33	38.46	0.63	78.16	1.50	64.00
F test	4.5		36.2		41.3	
LSD (0.05)	20.4		0.98		1.2	

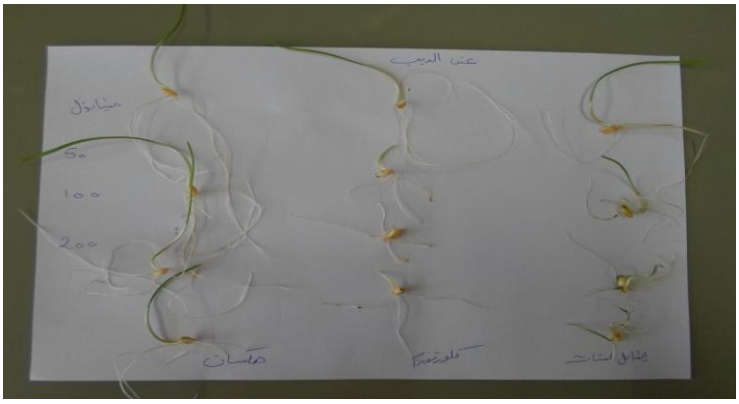

Figure 2. Effect of S. nigrum organic extracts on wheat seeds

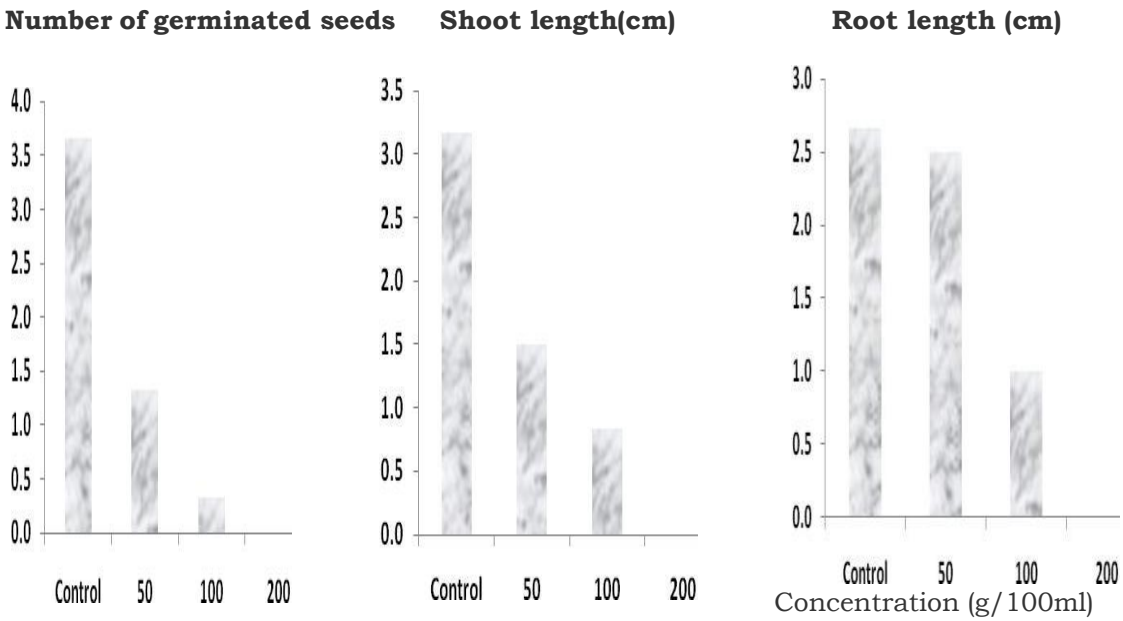

Figure 3. Effect of S. nigrum chloroform extracts on the Lolium grass plants

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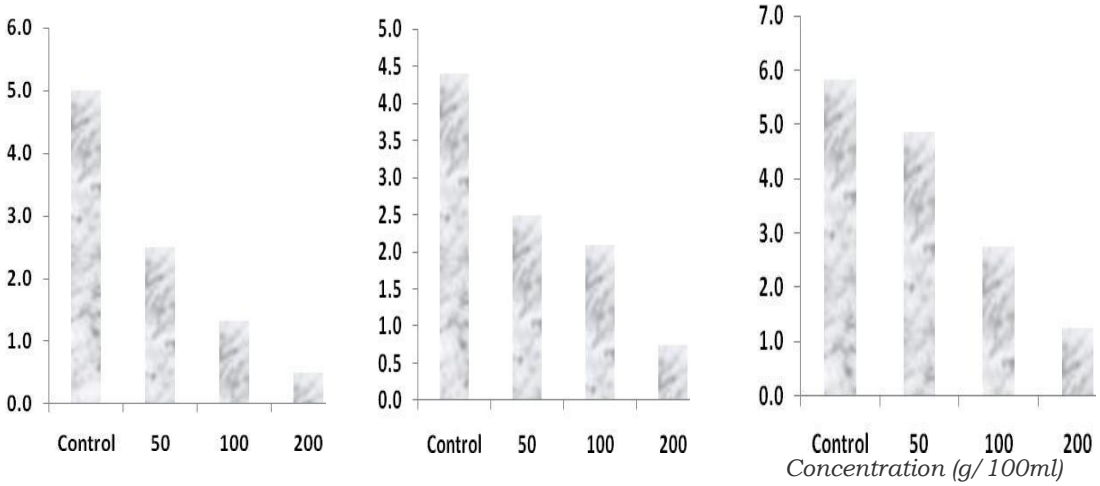

Figure 4. Effect of S. nigrum chloroform extracts on the wheat plants

Phytochemical Composition and Its Role in Allelopathy

Qualitative phytochemical screening revealed that all plant extracts contained phenolic compounds, flavonoids, saponins, tannins, and alkaloids in varying concentrations. *Solanum nigrum* showed the highest alkaloid and phenolic content, which may explain its stronger inhibitory effect. Phenolic compounds are well-known allelochemicals that affect seed germination by altering auxin metabolism and oxidative stress balance [1-19]. The presence of multiple allelochemical groups indicates a synergistic effect where combined compounds enhance the inhibitory potential [15]. Multiple compounds were isolated, and bioassays showed that several—including solasodine, chlorogenic acid, and caffeic acid derivatives—exhibited strong inhibitory effects on wheat seedling biomass. FTIR and MS confirmed functional groups and molecular weights, while UV spectra provided λmax data typical for phenolic and flavonoid compounds (Figures 5,6).

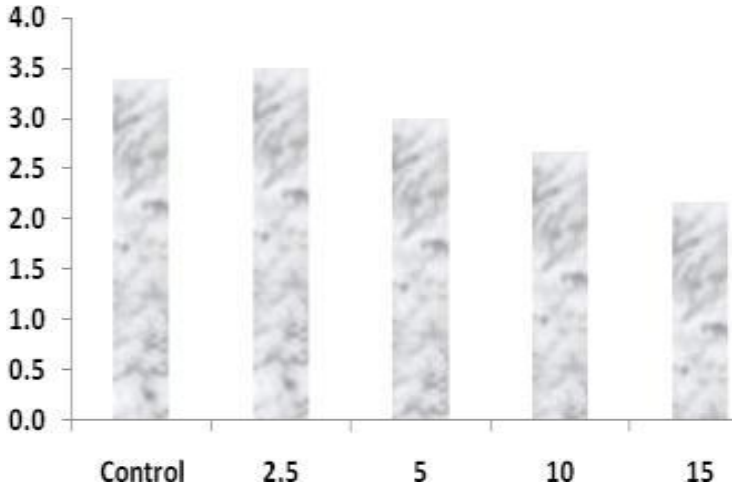

Figure 5. Representative HPLC chromatogram of ethyl acetate extract from Solanum nigrum showing major allelochemical peaks

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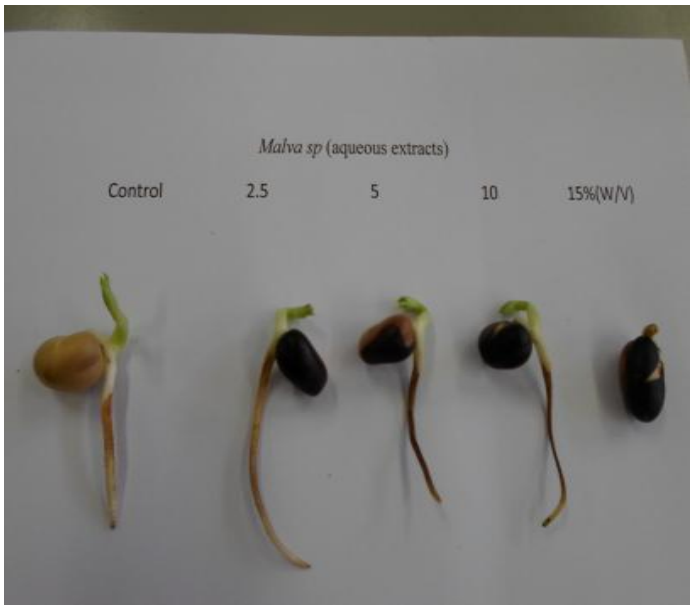

Figure 6. Mass spectrometry profile of solasodine with prominent fragment ions at m/z 414 and 396

Spectroscopic Characterization of Active Allelochemicals

Fractions with the highest bioactivity were analyzed using UV–Vis, FTIR, and NMR spectroscopy to identify their active constituents. The UV–Vis spectra of *Taraxacum officinale* and *Malva parviflora* extracts exhibited strong absorption bands at 220–280 nm and 310–340 nm, typical of conjugated aromatic systems and phenolic acids. The FTIR spectra showed characteristic peaks at 3400 cm^{-1} (O–H stretching), 1630 cm^{-1} (C=O stretching), and 1510 cm^{-1} (C=C aromatic), confirming the presence of phenolic and flavonoid structures (Figure 7, 8). The ^{1}H-NMR and ^{13}C-NMR spectra of the purified *Solanum nigrum* fraction indicated signals corresponding to steroidal alkaloids, particularly solasodine, with chemical shifts at δ 0.9–1.2 ppm (methyl protons) and δ 3.4–4.0 ppm (hydroxylated carbons). These findings align with previous reports [15-12] that allelochemicals such as phenolics and alkaloids exert biological effects by modulating plant signaling and redox pathways. A positive correlation was observed between phenolic content and inhibitory activity, suggesting that total phenolic concentration plays a key role in determining allelopathic strength. The bioassay results strongly support the chemical analysis, emphasizing the integrated action of multiple allelochemicals. This correlation highlights the ecological strategy of these species to suppress neighboring vegetation and secure a competitive advantage [20-21].

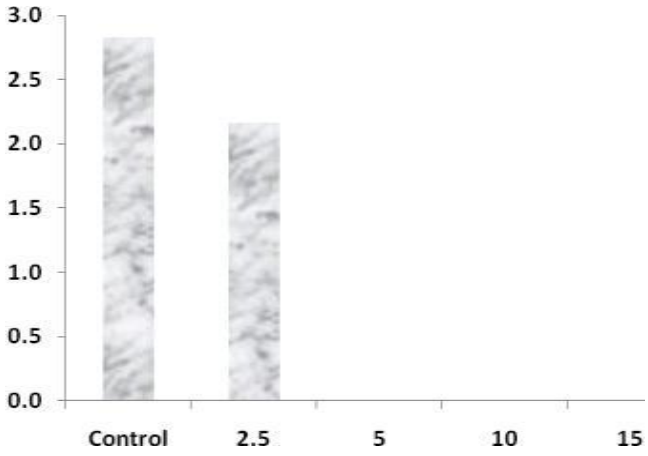

Figure 7. FTIR spectrum of purified cichoric acid derivative from Taraxacum officinale showing functional group absorption bands

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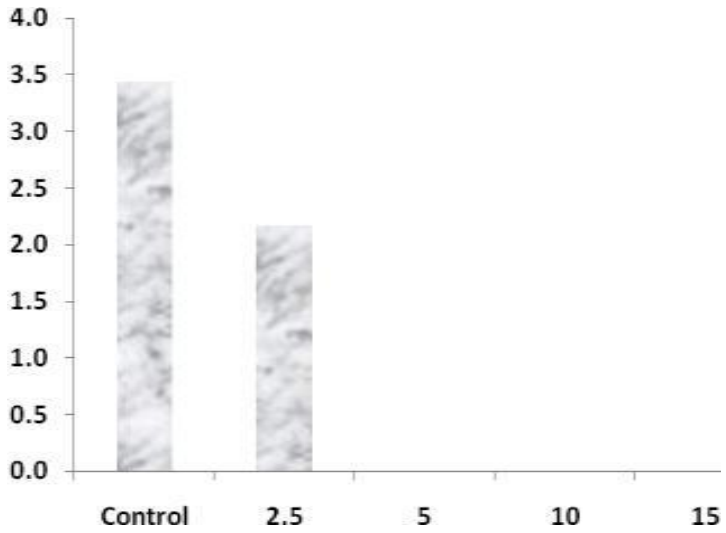

Figure 8. UV-Vis absorption spectrum of caffeic acid phenethyl ester showing λmax at 321 nm

Conclusion

The present study demonstrated that *Taraxacum officinale*, *Malva parviflora*, and *Solanum nigrum* possess significant allelopathic potential that adversely affects the germination and early growth of important crops. The observed inhibitory effects are attributed to secondary metabolites such as phenolics, flavonoids, and alkaloids identified through spectroscopic analyses. These results suggest that these species could be explored as potential sources of natural herbicidal compounds for sustainable weed management strategies.

Conflicts of Interest

The authors confirm that this research is free from any conflicts of interest.

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