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Role of secondary metabolites of wild marigold in suppression of Johnson grass and Sun spurge

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ABSTRACT

Objective: To analyze the wild marigold [(*Tagetes minuta* L.) (*T. minuta*)] leaf extract with respect to phytochemicals and allelopathic activity.
Methods: The aqueous extracts of *T. minuta* leaves at concentrations of 50%, 75% and 100% were prepared. Preliminary phytochemical analysis was carried out and then allelopathic effect of *T. minuta* on root length, shoot length, germination, fresh and dry weight of Johnson grass and Sun spurge was tested on filter paper and in soil.
Results: Qualitative phytochemical analysis showed the presence of alkaloids, tannins, saponins, flavonoides and terpenoids. The higher concentrations proved to be significantly effective in reducing almost all the parameters of Sun spurge and Johnson grass in filter paper bioassay. Supplemented with the soil, all concentrations of leaf extract showed reduction in germination, root and shoot growth, fresh and dry weight of Sun spurge; however, 100% concentration significantly reduced the germination of Johnson grass.
Conclusions: This study suggests that marigold allelochemicals can be used as an integrated weed management for the production of better crop yield.

1. Introduction

Organic production is facing severe weed infestation problem that lowers certain crop yields and their quality. Farmers and researchers are continuously trying to find the most effective and sustainable management of weeds in organic farming. Still, chemicals are used for controlling weed problems; however, in certain cases, weeds develop resistance towards herbicides and result in serious consequences. Cultural practices (crop rotations, cover crops, mulching), solarization, stale seed bed preparation, proper sanitation and composting, tactics to increase crop competitiveness and physical methods for minimizing the weeds on organic farms are proposed for effective weed management [1]. Hence, effective weed management is the only way to reduce weed control cost. Allelopathy also has a great deal with weed control management. Plants release certain chemicals into the environment which impede the germination, growth or development of other plants and it is called allelopathy [2]. The aqueous extracts of the shoots, rain leachates and the root exudates of plants are used in releasing allelochemicals [3,4]. So, phytochemicals can be released either through decomposition of plant residues, leaching, root exudation or volatilization [5].

The allelopathy can be used as a chemical warfare for interspecific and intraspecific competition in plants [6,7]. A number of plants produce poisonous substances so as to obstruct metabolic process of plants which may inhibit the growth of seed germination and seedlings [8]. The aqueous extracts and volatile chemicals of plants may affect as allelopathic nature on the root inhibition, hypocotyle growth, cell elongation and cell division of seedling tissues [9,10]. Such kind of activities can be considered as an ecological factor to establish the vegetation structure [11].

Allelopathy can be used as the best option for weed control, since there is a steady increase in herbicide resistance as well as increasing agricultural costs [12]. Nowadays, scientists are focusing on invasive species to identify and isolate phytochemicals with

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their potential use as herbicides. Besides, these species also affect the succession of species, allowing them to establish as pioneer species [13]. Nevertheless, the mechanism of allelochemicals and plant response is very selective in determining the action of such compounds [11]. Previously in arid and semi-arid areas of Pakistan, some of the studies have previously been carried out on allelopathic plants [14–17].

Wild marigold [*Tagetes minuta* L. (*T. minuta*)], locally called "Sadbarga", belongs to family Asteraceae. It is an annual plant with 50–150 cm height. In Pakistan, it is found at 3 000–11 000 feet height from above sea level and distributed in Punjab and Khyber Pakhtunkhwa Provinces of Pakistan. It is also found in Bagh and Azad Kashmir [18]. Due to the presence of specific phytochemicals, invasive species could have allelopathic attributes. Being an invasive species, it can play an important role in controlling weed populations. Therefore, the main objective of this study was to analyze the phytochemicals present in the wild marigold leaf extracts and to find out its allelopathic effects which may be attributed due to such phytochemicals.

2. Materials and methods

Fresh mature green leaves of *T. minuta* were collected from its natural habitats (Murree hill and Lehtrar, Kotli Sattian, Punjab, Pakistan). They were rinsed with distilled water, kept in paper bags individually and then put into the oven for drying at 60 °C. Subsequently after drying, it was crushed, sieved in 200 mm mesh size and preserved in glass bottles for further use. Voucher specimen (No. 2312) of plant and its seeds were deposited in the National Herbarium, Islamabad for the record.

2.1. Preparation of plant filtrate

Twenty grams of leaf powder were saturated in 100 mL of distilled water in a flask and were agitated for 24 h on orbital shaker at the room temperature. It was filtered by using Whatman filter paper No. 1 and the solvent was removed by using rotator evaporator. The extracts were weighed and stored in plastic vials and labeled. Further concentrations (*i.e.*, 50%, 75% and 100%) of this extract were also prepared.

2.2. Qualitative phytochemical analysis

The qualitative phytochemical screening was done by following the methodology of Edeoga *et al.* [19]. The plant diffusates were employed for the screening of alkaloids, tannins, saponins, flavonoids and total phenolics, coumarins and catechins.

2.3. Allelopathic evaluation

Allelopathic evaluation was carried out by using two screening methods as follows.

2.3.1. Aqueous extract on filter paper

This assay was employed to explore the growth inhibitory effects of water-soluble constituents [20]. Two layers of filter paper were placed in each sterilized glass Petri dish of 9 cm size. Weed seeds (10–20) were placed in each glass Petri dish and 5 mL of extracts at different concentrations (50%, 75% and 100%) were added per dish. Petri dishes were kept in replicates of 5 for each concentration. Distilled water was used as a control. Squash tape was used to seal the Petri plates and then incubated in the growth chamber for 10 days. This whole set of experiment was repeated thrice for each weed species.

2.3.2. Aqueous extract in soil

For this experiment, soil and sand were collected from the Research Farm of the National Agriculture Research Centre. The collected soil was crushed, air-dried and sieved through a 20 mm sieve in order to remove impurities. Soil (33%) and sand (67%) were mixed in pots in order to make the final mixture of 300 g. Different amounts of leaf powder (2 g, 4 g and 6 g) were thoroughly mixed with the soil and sand mixture. Weed seeds (10–20/pot) were sown in pots. For control, no extract was used. Three replications of each species at each concentration were used. Pots were placed in glass houses and watered according to daily requirements. Emergence of seedlings was recorded over 4 days of planting. Three treatments were used to investigate the effect of leaf extract on the growth and germination of testing weeds in soil.

2.4. Measurement of parameters

Fresh and dry weight, root and shoot length (mm), germination percentage and the growth percentage of root and shoot of all germinated weeds in all screening methods were recorded. Growth percentage of the root/shoot was calculated by the following formula:

$$\text{Growth percentage} = \frac{\text{Average length of root/shoot in particular treatment}}{\text{Average length of root/shoot in control}}$$

2.5. Statistical analysis

All the data were statistically analyzed in complete randomized block. Multiple comparison tests for means were also applied for those variables that showed statistically significant difference in ANOVA. Statistix 9 software was used for this purpose.

3. Results

3.1. Phytochemical screening

Standard tests used for phytochemical screening showed the presence of alkaloids, tannins, saponins, flavonoids and terpenoids in leaf extract, whereas resins, steroids, phenolics, coumarins and catechin were found to be absent in this extract. These results were in line with the findings of the previous investigation [21].

3.2. Allelopathic screening

3.2.1. Aqueous extract on filter paper

3.2.1.1. Root length

The results were presented in Table 1. All the three concentrations of a leaf extract were applied to filter paper, which affected non-significantly on the root length of Sun spurge (*Euphorbia helioscopia*), whereas 75% and 100% extract