Phytotoxic and Insecticidal Activities of Medicinal Plants of Pakistan, *Trichodesma indicum*, *Aconitum laeve* and *Sauromatum guttatum*

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Summary: The agrochemical potentials of ethanolic extracts derived from selected plants of Pakistan, including *Trichodesma indicum* (whole plant), *Aconitum laeve* (roots) and *Sauromatum guttatum* (corms, leaves and berries) were assessed through measuring their phytotoxic and insecticidal activities. All the extracts exhibited excellent herbicidal activity against *Lemna minor* L. and, except *A. laeve*, completely inhibited the plant growth at 500 μg/ ml. These extracts also showed good insecticidal activity with the highest mortality (80 % each) being observed with *S. guttatum* berries against *Bruchus pisorum* and *T. indicum* against *Rhizopertha dominica*. The results obtained were superior to most of similar studies reported in literature.

Introduction

The world human population is growing rapidly and is estimated to be 10 billion by the year 2050. To cope with the nutritional requirements of this population, more food and fiber would be needed from less land, water and use of labor. With the recent advances in plant biotechnology, this would not be a very difficult goal to achieve. However, a stiff obstacle in the enhancement of food production from the available resources is the infestation of crops by pests, weeds and diseases, which even increases with increasing intensity of production. The overall global loss of crop production due to these factors is estimated to be 42 %, which calls for urgent need for preventive measures.

Currently, the major eradication source for these crop production limiting factors is the use of synthetic chemical pesticides. However, some serious flaws are associated with the use of these synthetic pesticides including pest resistance and negative impact on natural enemies in addition to environmental and health related concerns [1-3]. These problems have resulted in the renewed interest in the development and use of botanical pesticides, which could be an appropriate and non-hazardous alternative to the currently used synthetic agrochemicals as the natural products, they are generally effective, biodegradable and thus pose less threat to the environment [4-5].

Numerous plants produce secondary metabolites, which provide them with protection against predation or infection and thus a source of their existence [6-7]. Similarly, a number of plants are allelochemicals to other plants [8]. The plants with aforesaid properties, their extracts or purified active constituents can act as insecticides or herbicides and thus could be the candidates for application for agricultural purposes.

To find some new natural sources of pesticides from botanical origin, we screened the crude extracts derived from medicinal plants of Pakistan, including *Trichodesma indicum*, *Aconitum laeve* and *Sauromatum guttatum* (Table-1). The present study is the continuation to our previous investtigations on the exploration of phytomedicinal and agrochemical potentials of medicinal plants of Pakistan [8-14].

Results and Discussion

Phytotoxic Activity

The herbicidal potentials of the extracts derived from the selected plants were evaluated against *L. minor* and the results are shown in Fig. 1. The results obtained indicated that most of the tested extracts possess tremendous herbicidal activity. The

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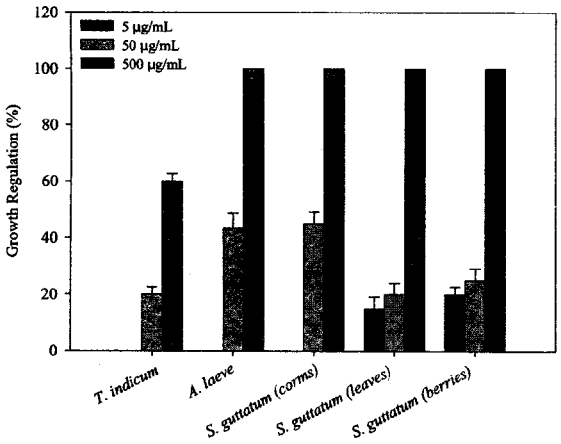

Fig. 1: Phytotoxic activity of ethanolic extracts derived from selected plants of Pakistan at 5, 50 and 500 µg/ ml against *L. minor*.

extract obtained from *A. laeve* and *S. guttatum* (corms, leaves and berries) have excellent phytotoxicity at the highest tested concentration (500 µg/ ml) and caused total (100 %) inhibition of the plant growth. At this concentration, extract from *T. indicum* also showed a good activity and inhibited the plant growth by 60 % (Fig. 1). At 50 µg/ ml, a moderate phytotoxic activity was observed for extracts from *A. laeve* and corms of *S. guttatum* and caused 43.3 and 45 % inhibition of *L. minor*, respectively. However, at this concentration only a weak inhibitory activity was observed for the extracts derived from *T. indicum* (20 %) and leaves and berries of *S. guttatum* (20 and 25 %, respectively). None of these extracts exhibited any significant herbicidal activity towards the tested plant at further lower concentration (5 µg/ ml). The berries and leaves of *S. guttatum* displayed 20 and 15 % activity respectively, while the extracts from *T. indicum*, *A. laeve*, and corms of *S. guttatum* were found to be completely inert towards *L. minor* at this concentration (Fig. 1).

Several researchers have screened the crude extracts of various plants for herbicidal activities using Lemna plants as model species. *Salvia moorcraftiana* has been reported to possess excellent herbicidal activity against *L. aequinoctials* with complete growth inhibition of this plant at 500 µg/ ml [8]. Similarly, *Abroma augusta* seed oil inhibited the growth of *L. aequinoctials* by 82 % at 500 µg/ ml [9]. Khattak *et al.*, [10] reported the herbicidal activity of *Aloe vera* (leaves) and *Mentha longifolia* (leaves and stem) against *L. minor* with the former plant exhibiting complete inhibition while the later showing ≥ 60 % inhibition of the model plant. *Curcuma longa* and *Alpina galanga* possess excellent phytotoxic activity against *L. minor* and caused complete inhibition of the tested plant at 1000 µg/ ml [15]. Similarly, several plant species were screened for allelopathic potentials against *L. aequinoctials* [16].

The results obtained in the current study indicated that the ethanolic extracts derived from the selected plants possess tremendous phytotoxic activity against *L. minor* (Fig. 1). These results are superior to most of the plant species [9,10,16] while comparable to the others previously reported for similar studies [9,10,15]. Therefore, the tested plants might be useful as natural herbicides and could be rich sources of bioactive agrochemicals.

Insecticidal Activity

The insecticidal activity of the crude extracts obtained from the selected plants was tested against *B. pisorum*, *T. castaneum* and *R. dominica* and the results obtained are given in Fig. 2. The extract derived from *T. indicum* showed excellent insecticidal activities against *R. dominica* and a moderate inhibitory activity against *B. pisorum*. This extract caused, respectively, 80 and 40 % mortality of these species. The same extract, however, did not display any inhibitory activity against *T. castaneum*. The extract from *A. laeve* displayed a moderate insecticidal activity against *R. dominica* causing 50 % mortality of this species (Fig. 2). This extract, however, was devoid of any inhibitory activity against *B. pisorum*, while only 10 % activity was observed against *T. castaneum*. The extracts derived from the berries of *S. guttatum* possessed excellent insecticidal activity against the *B. pisorum* causing 80 % mortality of this species. The same extract also exhibited a good (50 %) inhibitory activity against *R. dominica*, but only a weak (10 %) activity was observed towards *T. castaneum*. The extract from leaves of *S. guttatum* showed moderate to weak activities against the tested insect species inhibiting the growth of *B. pisorum*, *T. castaneum* and *R. dominica* respectively, by 40, 20 and 20 %. The extract of corms did not exhibit any activity against *B. pisorum* and *T. castaneum* while 50 % mortality activity was observed in case of *R. dominica* (Fig. 2).

B. pisorum is one of the most intractable pest problems of cultivated pea throughout the world [17] and is responsible for up to 40 % losses in seed

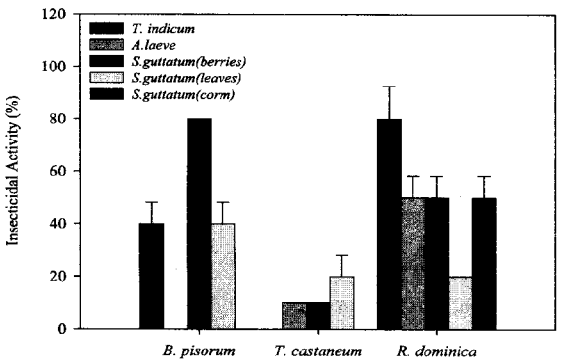

Fig. 2: Insecticidal activity of ethanolic extracts obtained from selected plants of Pakistan against *B. pisorum*, *T. castaneum*, and *R. dominica*.

yield [18]. Besides loss in crop yield, the infested pea seeds are also unsuitable for human consumption and for subsequent resowing [19]. Previously, we have reported the insecticidal activity of *P. emodi* [12], which displayed only a moderate (40 %) mortality against this species. During the current study, *S. guttatum* berries showed more significant inhibitory activity (80 %) while *T. indicum* and *S. guttatum* leaves displayed comparable activities (40 % each) to that of *P. emodi* (Fig. 2).

R. dominica is one of the most destructive beetle species of stored grain worldwide. The adults and larvae of this species can easily infest sound seeds and even can be developed and reproduce in grain with low moisture content. This species is now resistant to many traditional grain protectants [20], therefore, search for effective alternative substances especially; bioinsecticides against this species is the urgent need of the time. In an attempt, several plant species have been screened previously for the control of this insect. These include *S. moorcraftiana* [8], *P. emodi* [12], *A. augusta* seed oil [9], *A. vera*, *M. longifolia* [10], and *C. longa* [15]. However, none of these plant species displayed any inhibitory activity against *R. dominica*. The results obtained in the present investigations showed that most of the tested extracts have reasonable insecticidal activity with *T. indicum* having strong (80 %) inhibition against this pest (Fig. 2). The insecticidal activity of *T. indicum* is even better than the commercial pesticidal formulation, PyriSec® while the other extracts are comparable to it. PyriSec® contains diatomaceous earth with natural pyrethrum and piperonyl butoxide and displayed ~ 50 % mortality effect after 24 h of exposure [20].

During the present investigations, *T. indicum* and berries of *S. guttatum* have shown considerable insecticidal activity especially against *R. dominica* and *B. pisorum*, while *A. laeve* and leaves and corms of *S. guttatum* have shown good to moderate insecticidal activities (Fig. 2). Therefore, the studied plants could be new sources of bioinsecticides for stored products and field pest control.

In conclusion, the present study has identified several plant extracts, which could be candidates for the commercial botanical pesticide formulations. Moreover, the isolation, purification and investigation of the active principles responsible for the phytotoxic and insecticidal activities will be another landmark in the development of a verifiable application of these materials for the control of the pest vector. In a study on the phytomedicinal investigations of these plants, none of these extracts showed considerable brine shrimps cytotoxicity (IC_{50} > 200 μg/ ml) [14]. This declares the safe nature of these plants extract and thus provides nontoxic and environmentally friendly alternate sources of pesticides to the synthetic ones.

Experimental

Plant Material and Preparation of Extracts

The plants, *T. indicum* (whole plant) and *A. laeve* (roots) were collected in July from Swat, Pakistan and identified by Mehboob-ur-Rehman, Plant Taxonomist, Department of Botany, Government Degree College Matta, Swat. *S. guttatum* (tubers, leaves and berries) was collected from Mohmund Agency, Pakistan, in the month of July and its identification was confirmed by Professor Abdur Rashid, Department of Botany,. University of Peshawar, Pakistan.

In each case, the plant material was first shade dried and then pulverized into fine powder. All the powdered plant materials, except *A. laeve* were macerated with ethanol. The powder of *A. laeve* was first defatted with *n*-hexane and then the residue was extracted with ethanol. The crude extracts obtained were filtered and evaporated in *vacuu* to dryness. The percent yield of the extracts was calculated with reference to the dry mass of powdered plant material (Table- 1).

Table-1. Plants studied for pesticidal activities.

Botanical name	Common name(s)	Family	Plant material (s)	Extract yield (%)
Trichodesma indicum	Kulang kallen Nile karaji	*Boraginaceae*	Whole plant	6.25
Aconitum laeve	Jungli atis Maniree Zahar moora	*Ranunculaceae*	Roots	8
Sauromatum guttatum	Marjanay Sanp ki bhooti Voodoo lily	*Araceae*	Corms Leaves Berries	5.73 15 13.3

Phytotoxic Activity

Phytotoxic activity of the crude extracts was tested against the *Lemna minor* L. [21]. The extracts were dissolved in ethanol (20 mg/ ml), which served as stock solutions. Nine sterilized flasks, three for each concentration, were inoculated with 1000, 100 and 10 μl of the stock solution for 500, 50, and 5 μg/ ml, respectively. After evaporation of solvent, to each flask, 20 ml medium and 10 plants (*L. minor*), each containing a rosette of three fronds, were added. Three flasks containing only medium and plants (*L. minor*) were used as negative control. One other flask supplemented with solvent and reference growth inhibitor (paraquat, 0.9025 μg/ ml), served as positive control. All flasks were incubated in the growth cabinet for seven days after which the number of fronds per flask were counted and recorded. The results were interpreted by analyzing the growth regulation in percentage calculated with reference to the negative control with the help of the following formula:

$$\text{Growth regulation (\%)} = \frac{100 - \text{Number of fronds in test flasks}}{\text{Number of fronds in negative control}} \times 100$$

Insecticidal Activity

The insecticidal activity of plant extracts was determined by direct contact application using filter paper [22]. The extracts (200 mg) dissolved in methanol (3 ml) were applied to filter papers (90 mm diameter). After drying, each filter paper was placed in petri dish and 10 adults each of *Bruchus pisorum*, *Tribolium castaneum*, and *Rhizopertha dominica* were placed in each petri dish and covered with a lid. A check batch was treated with solvent for determination of solvent effect. A control batch was kept for the determination of environmental effects. Another batch was supplemented with reference insecticide (permethrin, 235.71 μg/ cm^2). All these were kept without food for 24 h after which mortality count was done. All treatments were replicated three times and reported as the average. For calculation of % mortality the following formula was used:

$$\text{Mortality (\%)} = \frac{100 - \text{Number of survival in samples}}{\text{Number of survival in control}} \times 100$$

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