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Research Article
Bio-controlling Effect of Leaf Extract of *Tagetes patula* L. (Marigold) on Growth Parameters and Diseases of Tomato

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Abstract

Background: The genus *Tagetes* (Asteraceae) is native to Americas but some of its members (in particular *T. erecta* and *T. patula*) commonly known as marigolds were naturalized in the old world (India, North Africa and Europe) as early as in 16th century. The flowers of French marigold (*Tagetes patula* L.) are widely used in folk medicine, in particular for treating inflammation-related disorders. **Materials and Methods:** This study investigated the potential use of marigold (*Tagetes patula* L.) flower aqueous flower extract by spaying method on tomato plants on a weekly basis and the data of growth, yield and disease of tomato pants were observed from 10th day onwards under field condition. **Results:** The marigold flower extract showed significant increase in shoot height, number of branches, number of leaves, number of buds, number of flowers and number of fruits of tomato plant, while significant reduction in various diseases of tomato plants over control at probability level ***$p<0.001$. The percentage of reduction of disease was calculated after the spray of marigold flower aqueous extract on plants. The marigold flower extract was found effectively in controlling canker (62.82%), early blight (61.53%), wilt (18.42%), fruit spot (27.41%), blossom end rot (50.43%) and sun scald (26.44%) in comparison to controls under field condition. **Conclusion:** The findings are in line with the bio-controlling properties of marigold preparations as bio-pesticide confirmed in growth and yield of tomato plants. Thus, marigold can contribute in reducing use of chemical pesticides and act as a good alternative to synthetic pesticides.

Key words: Bio-pesticide, diseases, growth parameters, marigold extract, tomato

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Botanical insecticides possess a spectrum of properties including insecticidal activity, repellence to pests, antifeedancy, insect growth regulation, toxicity to nematodes, mites, snail and slugs and other pests of agricultural importance. Also they possess antifungal, antiviral and antibacterial properties against pathogens. Bio-pesticides of botanical origin have become the focus of attention today for facing the nematode problems in an eco-friendly manner[1,2]. The use of botanical pesticides is now emerging as one of the prime means to protect crops. These bio-chemicals are referred to collectively as "botanicals" which have advantages over broad-spectrum conventional pesticides. They affect only target pest and closely related organisms are effective in very small quantities, decomposed quickly and provide the residue free food and a safe environment to live. When incorporated into integrated pest management programs, botanical pesticides can greatly decrease the use of conventional pesticides or can be used in rotation or in combination with other insecticides, potentially lessening the overall quantities applied and possibly mitigating or delaying the development of resistance in pest populations.

Since most of them generally degrade within a few days and sometimes within a few hours, these bio-pesticides are being frequently used for the management of phytoparasitic nematodes[3]. In India, botanical pesticides are available in many plants for which deep search and testing is required as many of them are still unexplored[4,5]. As they are available in bulk, they are expected to be cheaper in comparison to synthetic chemicals. Moreover, the farmers can prepare their crude extracts for their use in field. Many plants and plant products when applied in soil are known to cause reduction in the nematode population below damaging level. In few cases plants have been found to be actually antagonistic towards nematodes and some have shown to produce toxic materials inhibitory to nematodes[5,6].

There is increasing interest in the development and adoption of environmentally friendly tactics for managing nematodes, particularly as fumigants and other chemical nematicides become more limited[7]. Current alternative practices most often used include growing nematode-resistant varieties and rotating with non-host crops. However, the wide host range of some nematode species and the unavailability of resistant varieties limit the use of crop rotation in several production systems. Another environmentally favorable approach that has been the subject of much research and is closely aligned to sustainable cropping principles is the growing of nematode resistant or antagonistic plants.

Tagetes is a multipurpose plant having ornamental, ritual, medicinal, anthelmintic, insecticidal, colorant, food and forage applications[8,9]. In particular, flowers and entire herb of *Tagetes patula* L. (French marigold) are used for preparing ethno-botanical remedies against rheumatism, stomach and intestinal problems, kidney and hepatic disorders, fever and pneumonia[8,10]. Marigolds' repressive impact on nematodes has been documented for over 50 years[11]. In addition to being insecticidal, fungicidal activity of *Tagetes* against *Helminthosporium oryzae* was reported[12]. Various *Tagetes* oils appeared to inhibit Gram-positive bacteria and fungi[13].

Large number of studies has been undertaken in the laboratory against the causal organism affecting the tomato plant. However, the effectiveness of the marigold extracts on individual crops in different agro-climatic zones and cultivars in field condition have not been investigated much. Hence, the objective of the study was to determine to efficacy of aqueous flower extract marigold for controlling some important diseases like canker, early blight, fruit spot, blossom end rot, wilt and sun scald as well as its effect on growth and yield of tomato plant.

MATERIALS AND METHODS

Preparation of experimental plots and plantation: The grasses and weeds of experimental plot were removed and the land ploughed to a depth of 15-30 cm and several holes with the dimension of 30×30×30 cm were made every 150 inches intervals. In each hole, required amount of cow-dung and organic fertilizer at the rate of 6-12 t ha^{-1} was applied. Optimum spacing 90×60 cm with one plant/hill was maintained. About 10 days old tomato seedlings were planted.

Preparation of aqueous marigold flower extract: Hundred grams from each of the dried, powdered flowers were weighed and were mixed in 1000 mL distilled water. Then the solution was boiled, cooled and filtered through the cheese cloths followed by filtration by the Whattman No. 1 filter paper. Then filtrate was kept under normal room temperature and sprayed on the plants on a weekly basis from 10th day onwards.

Growth, yield and disease parameters: Morphological measurements of tomato plants (*Solanum lycopersicum* L.) were taken during 10-120 days with 10 days intervals after transplantation till harvest time. The growth parameters were taken into consideration and they are as follows: Shoot

height, number of leaves, number of buds, number of flowers and number of fruits. The percentage of infection (canker, early blight, wilt, leaf spot, fruit spot, blossom end rot and sun scald) were investigated after 4 weeks of transplantation. Numbers of diseases were calculated as percentage and diseases per plant.

Preparation of crude extract: The collected flowers were shade dried under normal environmental condition and then ground into uniform powder using Thomas-Wiley machine. The powdered flowers (50 g) were extracted with distilled water by using Soxhlet extraction apparatus for 10-12 h. Then collected solutions were filtered through Whattman No. 1 filer paper. The extracts were evaporated to dryness under reduced pressure at 90°C by Rotary vacuum evaporator to obtain the respective extracts and stored in a freeze condition at -18°C until used for further analysis.

RESULTS AND DISCUSSION

Growth and yield: The experiment was conducted on growth and yield of tomato cultivation of marigold extract and the results on effectiveness of various treatments were described. There was significant increase in the plant height, number of branches and leaf number at all growth stages from 10-90 days in marigold extract treatment in comparison to control (Table 1). Number of buds from 40-80 days, number of flowers from 50-90 days and number of fruits 90-120 were recorded (Table 2-4). All measured parameters gave significant differences from their respective controls at probability levels (*p<0.05, **p<0.01 and ***p<0.001). Marigold aqueous flower extract showed a promotive effect on shoot lengths (75.87%), branches (27.42%) leaf numbers (17%), number of buds (42.71%), number of flowers (54.96%) and number of fruits (66.21%) with increasing time compared to untreated or control ones (Fig. 1). The promotive effect could be due to flavonoids patuletin, quercetagetin, carotenoid lutein and quercetin and their derivatives which act as a strong antioxidantive and cyto-protective activity reported by Hooks *et al*.[14]. Growth stimulating effect of 10 medicinal plant extracts (*P. pinatta*, *A. marmelos*, *A. indica*, *B. campestris*, *P. nigrum*, *E. tirucalli*, *V. negundu*, *A. conyzoides*, *T. patula* and *Z. jujube*) on *Lycopersicum esculentum* L. have been observed[15,16]. Similar experiment carried out by Okunlola and Ofuya[17] showed the effect of *A. indica* and *Piper guineense* on the growth and yield of jute under sole and mixed cropping. All growth parameters increased in comparison to control. In another experiment ethanolic extracts of *Melia azedarch*, *Eucalyptus robosta* and *Sapium sebiferum* had no significant influence on growth and development of soybean seedlings[18]. Effect of tea seed extracts on growth of beet, mustard, oat and barley were studied. Different concentrations of these extracts increased the growth, yield and biomass of the crops. The growth stimulating effect is not exclusively by its adverse effect on pathogen or by an increase in nutrient uptake. However, substances with hormone like properties can stimulate of effect biomass allocation in plants. In addition to hormones,

Table 1: Effect of *T. patula* aqueous extract on plant height, No. of leaf and No. of branch *Lycopersicon esculentum* L.

Parameters	10 days	30 days	50 days	70 days	90 days
Plant height (Mean±SEM)					
Control	6.30±0.49	15.7±1.03	16.4±1.14	29.7±0.88	37.3±1.11
Tagetes patula	4.00±0.25**	11.8±0.78*	31.1±1.80***	45.6±2.68***	65.6±2.98***
No. of leaf (Mean±SEM)					
Control	11.4±1.04	26.4±2.91	42.5±3.44	63.4±4.14	68.0±9.45
Tagetes patula	10.4±0.76***	25.2±2.10*	42.6±2.10*	78.6±3.45**	79.8±2.56***
No. of branches (Mean±SEM)					
Control	3.60±0.16	6.30±0.30	7.80±0.51	15.0±1.41	17.5±1.52
Tagetes patula	12.70±3.50**	13.90±2.81**	10.90±2.07*	17.10±3.70*	22.3±5.07***

Probability levels *p<0.05, **p<0.01 and ***p<0.001

Table 2: Effect of *T. patula* aqueous extract on number of buds of *Lycopersicon esculentum* L.

Buds (Mean±SEM)	40 days	50 days	60 days	70 days	80 days
Control	2.80±0.38	4.00±0.68	5.70±0.80	7.90±0.31	10.13±0.58
Tagetes patula	6.70±0.85***	11.90±1.51***	11.70±0.42***	12.10±0.58***	14.70±2.79***

Probability levels ***p<0.001

Table 3: Effect of *T. patula* aqueous extract on number of flowers of *Lycopersicon esculentum* L.

Flowers (Mean±SEM)	50 days	60 days	70 days	80 days	90 days
Control	4.1±0.92	5.2±1.05	7.9±0.75	9.5±1.83	13.1±1.74
Tagetes patula	11.4±0.80***	13.2±0.55***	14.2±1.09***	14.7±0.78***	20.3±1.94***

probability levels ***p<0.001

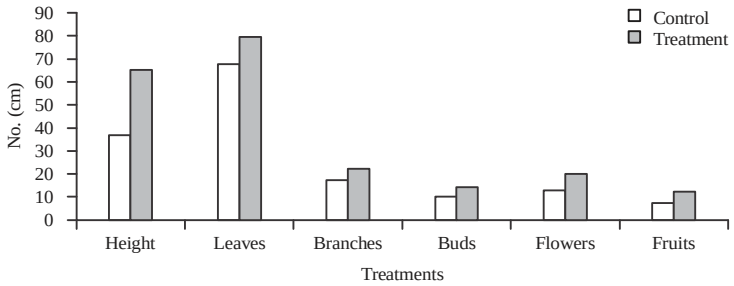

Fig. 1: Graph showing growth parameter of *Lycopersicon esculentum* in control and *Tagetes patula* L., extract treatment

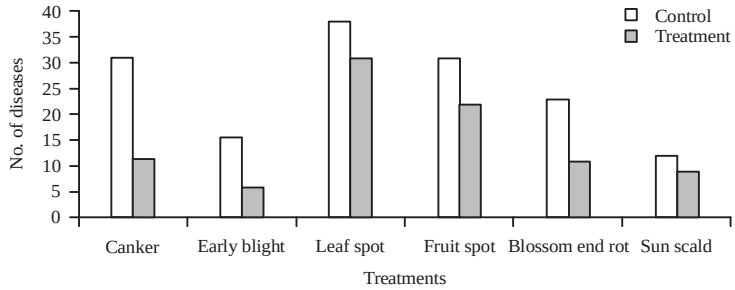

Fig. 2: Graph showing number of diseases of *Lycopersicon esculentum* in control and *Tagetes patula* L., extract treatment

Table 4: Effect of *T. patula* aqueous extract on number of fruits of *Lycopersicon esculentum* L.

Fruits (Mean±SEM)	90days	100 days	110 days	120 days
Control	3.00±0.39	4.20±0.67	5.00±0.78	7.40±0.42
Tagetes patula	6.30±1.43***	7.40±1.39***	10.10±1.41***	12.30±1.59***

Probability levels ***p<0.001

medicinal plant extracts contain saponins and polyphenols which could be the active compounds causing the effect on growth[19].

Diseases: Because of its fleshy nature, tomato fruit is attacked by a number of insect pests and diseases. Pathogenic diseases develop through soil borne and above ground infections and in some instances are transmitted through insect feeding. Major tomato diseases include those that attack the root system (fusarium wilt, verticiliun wilt, bacterial wilt, rhizoctonia), above ground stems and foliage (early blight, leaf spot, bacterial canker, late blight) and fruit (bacterial spot, bacterial speck, anthracnose)[20]. Thus, the disease control programme is important at each stage of growth (Tomato diseases and disorders). In our findings we observed better controlling effect on these major diseases of tomato plant such as canker, early blight, wilt, fruit spot, blossom end rot and sun scald in comparison to controls and gave significant differences from their respective controls at probability levels (*p<0.05, **p<0.01, ***p<0.001) under field condition (Table 5, Fig. 2).

Canker caused by *Clavibacter michiganensis* subsp., *michiganensis* (Cmm) is a serious pathogen of tomatoes which causes serious losses in some tomato plantings. It is often first seen as dark, necrotic lesions at the margins of older leaves. This disease can cause lesions or cankers on any portion of the plant, including the fruit or it can result in a general wilt or decline of the plant[21,22]. In our field experiment marigold reduced canker disease by 62.82% in comparison to control. Different combinations of natural treatments could give better effects against bacterial canker (*Clavibacter michiganensis* subsp., *michiganensis*) and bacterial speck (*Pseudomonas syringae* pv., tomato) of tomato such as natural compounds[23], plant extracts[24], essential oils[25] and natural acids[26]. It was studied that the essential oils from *T. spicata* and *O. syriacum* had the highest inhibitory activity against *Clavibacter michiganensis* subsp., *michiganensis* corresponding to 83.6 and 82.8% increase in the zones of inhibition over the control[27].

Early blight caused by the fungus *A. solani* is common foliage diseases which first appears in the older leaves and are characterized by irregular shaped brown spots with concentric rings[28,29]. These infections often occur near the stem of the tomato fruit. We observed an effective reduced early blight (61.53%) in comparison to control. Gomez-Rodriguez *et al.*[30]

Table 5: Effect of *T. patula* aqueous extract against different Diseases of *Lycopersicon esculentum* L.

Parameters	40 days	50 days	60 days	70 days	80 days
Canker (Mean±SEM)					
Control	23.0±2.91	28.5±1.64	26.8±0.96	25.6±2.75	31.2±2.49
Tagetes patula	26.3±2.40*	22.1±1.62**	20.8±1.66**	15.5±1.40**	11.6±2.20***
Early blight (Mean±SEM)					
Control	14.0±1.19	12.4±0.96	14.2±1.44	13.8±0.79	15.6±1.16
Tagetes patula	12.5±1.55*	9.33±1.33**	9.00±1.00**	7.25±0.45**	6.00±0.44**
Leaf spot					
Control	67.4±2.86	66.7±6.94	49.1±4.06	45.0±7.59	38.0±4.16
Tagetes patula	55.2±3.52**	46.8±4.39**	42.6±3.45*	34.7±2.96*	31.0±3.02*
Fruit spot (Mean±SEM)					
Control	29.0±2.08	23.5±5.50	29.0±3.00	30.0±0.00	31.0±7.00
Tagetes patula	27.5±3.50**	25.5±3.50*	23.5±5.50**	23.0±7.00**	22.5±10.5**
Blossom end rot (Mean±SEM)					
Control	15.1±1.62	16.8±2.83	21.6±2.30	21.8±2.06	23.0±2.91
Tagetes patula	14.3±2.14*	14.1±2.40*	13.2±2.22**	13.0±2.12**	11.4±2.02**
Sunscald (Mean±SEM)					
Control	10.5±1.41	10.3±0.76	11.5±1.01	11.6±0.70	12.1±0.40
Tagetes patula	19.9±0.90**	18.0±0.77**	16.5±1.07**	13.3±0.95**	8.9±0.84**

Probability levels *p<0.05, **p<0.01, ***p<0.001

found that intercropping tomato with *T. erecta* reduced early blight (*Alternaria solani*) of tomato in three ways: (1) Allelopathic effect on *A. solani* development, (2) Reduced humidity levels below those conducive to the pathogen requirement and (3) Behaved as a physical barrier against spore dispersal[30,31]. Intercropping marigold for nematode management also appeared to reduce numbers of aphids and whiteflies and resulted in lower levels of virus in tomato[31]. In addition to nematicidal, bactericidal, fungicidal activities, other values of *Tagetes* reviewed by Vasudevan *et al*.[9] include medicinal importance, tea making and food coloring.

Wilt is caused by the soil borne fungus *Fusarium oxysporum* f. sp., *lycopersici*. The flower extracts of *Tagetes patula* L., substantially reduced the number of infected leaves and number of lesions on foliage and curtailed disease development, which in turn, protected flowers and capsules from infection[32]. Similarly this result also showed a positive effect on reducing fusarium wilt by 18.42%. The results obtained from El-Khallal[32] and Croxton *et al*.[33] who reported that the growth rate of shoot and root was markedly inhibited in tomato seedlings in response to fusarium wilt diseases. This further strengthens our field experiment on wilt control of tomato by aqueous neem extract. Leaf spot caused by the fungus *Septoria lycopersici* which are noticeable by small, circular spots on the upper surface of the lower leaves.

Fruit spot caused by *Pseudomonas syringae* pv. is the most common disease in tomatoes. Spots on tomato fruit are not very deep. They can be cut out and the tomato can be eaten[34]. Suppression of bacterial spots of tomato with foliar sprays of compost extract under green house and field condition was investigated. The population of infected leaves was reduced significantly by extracts prepared from cow manure[35]. In the present study marigold flower aqueous extract treated tomato plants showed significantly better performance over control by 27.41%.

Blossom end rot which is caused by a calcium deficiency that is related to wide fluctuations in available moisture which can be caused by excessive use of nitrogen. Brownish black spots on the blossom end of the fruits which may gradually increase in size invade the lesion and cause complete rotting of the fruit[34]. Reduction in incidence of blossom end rot of tomato by foliar application of plant extract mixed with Ca and sugar ester reduced the incidence of blossom end rot in comparison to control[36]. However, marigold extract only reduced BER by 50.43% without any addition of minerals and micro nutrients.

Sun scald appears as light patches on green or ripening fruit. As the patches grow, they may blister and may become grayish-white. Affected sun scald tomatoes can develop black mold (Sun burn or sun scald-peppers and tomatoes). We noticed in our experiment where the foliar application of marigold flower extract reduced 26.44% of sun scald in comparison to controls under field condition.

The crude extracts of marigold flowers and purified fractions containing flavonoids patuletin, quercetagetin, quercetin and their derivatives, as well as the carotenoid lutein independently or in combination may be responsible for the broad range of medicinal properties of marigold plant which exhibit an extra ordinary array of pharmacological activities. Research data from some studies appears not to support

the idea that nematicidal activity is associated mainly with functioning marigold roots and that (semi) endo-parasitic nematodes are primarily affected. Siddiqui and Alam[37] found that all above ground parts (flower, leaf, stem) of *T. lucida*, *T. minuta* and *T. tenuifolia* when incorporated into the soil reduced root galls caused by *M. incognita*, population densities of root-knot and reniform nematode (*R. reniformis*) on tomato and eggplant and stunt nematode (*Tylenchorhynchus brassicae*) on cabbage and cauliflower in pot experiments. In a later study, the same researchers compared the nematicidal activity of different parts (leaf, flower, seed and root) of *T. lucida* on reniform, lance (*Hoplolaimus indicus*) and spiral (*Helicotylenchus indicus*) nematodes and reported that although all parts of *T. lucida* were deleterious to the nematodes tested, flower extracts had the strongest nematicidal activity, followed by seed, leaf and root extracts[38]. Similarly, hatching of *M. incognita* eggs was inhibited more strongly by water extracts from flowers. Hassan *et al.*[39] also found that leaf extracts of *T. patula* were toxic to *M. javanica* juveniles in a petri dish test. These findings would suggest that the aerial parts are more toxic than the roots. Since Bakker *et al.*[40] and Gommers[41] reported that photo-activation is necessary to trigger the nematocidal activity of α-terthienyl, Siddiqui and Alam[38] proposed that the lower toxicity of marigold roots was due to the fact that they grow in the absence of light. This potential has already been demonstrated in a study where white cabbage intercropped with another species of *Tagetes* (*T. patula nana* L.) suffered significantly less cabbage aphid infestation when compared with a mono-cropped cabbage and tomato[42,43]. Tibugari *et al.*[44] studied the effectiveness of the aqueous extracts of garlic, castor beans and marigold in the biocontrol of root-knot nematode in tomato plants.

The results of the pesticidal and phytochemical screening of higher plants indicates that the plants are endowed with pesticidal properties that can be harnessed cheaply for use in agricultural, pharmaceutical and other related fields in an eco-friendly manner by replacing synthetic pesticides.

CONCLUSION

The current study demonstrated that the crude extract of *Tagetes patula* L., obtained using water as a solvent is an effective as bio-controlling preparation against the various diseases of tomato plants. It not only had reduced the percentages of disease level but also increases the growth and yield rates of tomato plants which confirmed its strong bio-efficacy property in field condition. These results augur well for the practical use of marigold as a source of effective and easily available botanical pesticide to resource-poor farmers against various diseases in tomato production. Based on the results from this study, we are planning to undertake a field-based experiment to evaluate *Tagetes patula* L., insecticidal activity against not just tomato but the whole insect herbivore complex of other commonly grown vegetables in India. We also plan to study the insecticidal effects of whole plants of *Tagetes patula* L., when grown in an intercropping system with vegetables. To make their use more meaningful, economical, feasible and environmentally safe, research efforts are needed to find out the toxic components present in them and their mode of action. For the conservation of biodiversity aiming to maximize food production and minimizing health hazards, botanicals may stand as the most promising source of bio-active products of plant origin.

SIGNIFICANCE STATEMENTS

There is an increasing interest in the development and adoption of environmental friendly pesticide for management of various plant diseases. A plethora of research has been conducted on the use of marigold for nemato suppression. Although plant parts and extracts of marigold have been experimented on nematocidal properties effect on fungicidal and bacterialcidal diseases have been only partially investigated. Due to presence of metabolic toxins further research on this plant help in discovering new chemical classes of antibiotics that could serve as selective agents for the maintenance of plant and human health and provide biochemical tools for the study of infectious diseases.

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