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# EVALUATION OF PLANT EXTRACTS AGAINST POST-HARVEST ROTTING FUNGI FROM TOMATO FRUITS

**SHER WALI KHAN[1], AZHAR HUSSAIN[2], QAMMAR ABBAS[2], GHULAM RAZA[3], AMJAD ALI[2] AND SHAHNAWAZ QASIM[2]**

[1]*Department of Biological Sciences Karakoram International University Gilgit, Pakistan*
[2]*Department of Agriculture and Food Technology Karakoram International University Gilgit, Pakistan*
[3]*Department of Biological Sciences University of Baltistan Skardu, Pakistan*
[*]*Corresponding author's email: dr.sherwali@kiu.edu.pk; azhar.hussain@kiu.edu.pk*

**Abstract**

The most common postharvest concern is fungus-caused tomato fruit rot. Produce rotting occurs between harvest and eating due to the fungus. The goal of this study was to see how well five plant extracts worked against the three most common rotting fungus species that cause tomato fruit rot (*Aspergillus flavus*, *Rhizopus stolonifer* and *Fusarium oxysporum*). Results showed that all plant extract exhibited antifungal activity ($p$<0.05) against tested pathogens. The radial colony fungal growth is decreased with increasing concentrations of the plant extracts. All plant extract at 2000ppm concentration showed ≥80 % mycelial growth reduction for all the three rotting fungi respectively. Overall *Aspergillus flavus* is more sensitive rotting fungi against plant extracts followed by *Rhizopus stolonifer* and *Fusarium oxysporum*. Plant wise highest antifungal potential has appeared in *Artemisia absinthium* and *Artemisia brevifolium* while least in *Salvia nubicola*. The outcome of the current endeavor indicates that plant extract can be used to control tomato rot fungal species.

**Key words:** Antifungal activity, Plant extracts, Tomato fruit rot, Pathogenic fungi, Gilgit.

## Introduction

Tomato (*Solanum lycopersicum* L.) is a short-lived perennial herbaceous plant belonging to the family Solanaceae. It is grown all over the world, with high nutrition with diverse uses (Afroz *et al*., 2008; Ewulo *et al.*, 2008). It is highly perishable, short shelf life and is susceptible to post-harvest spoilage microorganisms. Approximately 50 % of fresh produce is lost from farm to table (Oyeniran, 1988; Oyekanmi (2007). Tomatoes fruits are attacked by many kinds of pathogens including fungi, bacteria, nematodes and insects etc. Among tomato fruits rot caused by fungi distresses this crop, causing a huge decrease in yield and a greater loss of fresh produce. Kader, 1992 reported that main cause of tomato deterioration is due to fungal activity. This problem is more severe in developing countries (Jones *et al*., 1993). Most predominant fungal species triggering postharvest problem i.e *Aspergillus* spp., *Alternaria* spp., *Botrytis cinerea*, *Monilinia lax*, and *Rhizopus stolonifer* and *Fusarium* species (Ogawa *et al*., 1995). Most pathogens contaminate tomato fruits by physical injury or physiological breakdown. Initially, pathogens can contaminate superficially (Kader, 1992). The physiological nature of tomato fruit is supportive for the spread of pathogen under favourable environmental conditions (high humidity and temperature) as a result, heavy post-harvest losses (Idah *et al*., 2007). Some reports indicate that post-harvest wastage of tomatoes fruit is ≥ 50 % (Anon., 2002). Zaldivar (1991) stated that post-harvest losses are 28-42% worldwide, and in the less developing countries the losses ranged from 15-50% or 15-60%. This means fifty percent of food loss before reaching the consumers. To minimize these losses the use of synthetic chemicals is most common, promising results oriented but they caused human and environmental effects (Jobling, 2000; Sharma *et al*., 2005; Azhar *et al*., 2014). Several studies proved that synthetic chemicals effectively control the post-harvest disease/decay of fruits and vegetables (Adaskaveg *et al*., 2004; Kanetis *et al*., 2007). However, these chemicals impart residual effects on fruit, the environment and human health. Therefore it is a dire need to develop alternative methods that have minimum risk and ecofriendly. Now the application of plant extracts to control fruits decay and extension shelf life has gained more attention (Kamlesh *et al*., 2007; Chandra & Mahesh, 2013). Plant-based active component/natural products have the significant potential to substitute synthetic chemical fungicides. Plant-based extracts or essential oils are the key sources of antifungal activity against the fungal pathogen and important sources of biopesticides (Anuradha & Bandopadyay, 2008; Combrinck *et al*., 2011). The present investigation aimed to evaluate the effectiveness of different plant extract against selected tomato rotting fungi.

## Materials and Methods

**Collection of fruit sample:** Tomato rotting fruits were collected from different survey areas of District Gilgit. These fruits were packed in bags and transferred to the laboratory for the isolation and identification of microorganisms (Fig. 1).

**Fungal pathogens isolation and identification:** To isolate associated fungal tomato fruits samples were treated with sodium hypochlorite for 60 seconds then rinsed distil water in three changes. After drying the infected fruits were cut into small pieces; place on streptomycin sulfate (50 mg $l^{-1}$) amended potato dextrose agar medium (PDA) These Petri dishes were kept at 27 ± 1°C for a week for fungal growth. Fungal species were identified microscopically as described by Barnett & Hunter 1998 and Dugan, 2006. Three dominant tomatos rot fungal species *Aspergillus flavus*, *Rhizopus stolonifer* and *Fusarium oxysporum* were purified. Different concentrations of plant extracts were evaluated against selected fungal species.

Fig. 1. Isolation of tomato rotting fungi "samples collected from different areas of District Gilgit".

**Preparation of plant extracts:** The leaves of five plants namely *Salvia nubicola Uretica dioica Delphinium. brunonianum Artemisia absinthium* and *Artemisia brevifolium* were collected from different locations of Gilgit-Baltistan. The plant leaves were air-dried (27± 1°C) for10-12 days and grounded to make a fine powder. 50 g of powder of each plant extract were mixed with ethanol and distill water (20: 80 v/v) for 20 min then left samples in dark glass bottles for 3 days. Finally, by using cheesecloth sheets filter plant extract and expose to 60°C in a water bath for 30 min for ethanol evaporation. From these samples different concentration of ppm solution (400-2000 ppm) was prepared and assessed against targeted fungal species.

**In vitro bioassay:** The food poison technique was used to evaluate the antifungal efficacy of plant extract (Manmohan & Govindaiah 2012). Five treatments (400-2000ppm) along with control with three replications was design in this experiment as well as PDA was as nutrient medium. The actively growing culture of isolated rot fungi were cut by gel cutter and aseptically transfer to each Petri dish containing the poisoned solid medium. All the experimental materials were nurtured at 27± 2°C for one week. After the incubation period, the efficacy of plant extract was quantified in term PGI (percentage growth inhibition) according to formula (Taskeen *et al*., 2011; Sallam & Kamal 2012).

$$GIP = \frac{MGC - MGT}{MGC} \times 100$$

GIP = Growth inhibition percentage
MGC = Mycelial growth in control
MGT = Mycelial growth in treatment

**Results and Discussion**

Experimental results of the current study indicate that plant extract with different concentrations significantly reduced the mycelial growth of respective tomato rot fungi. (Table 1) present the antifungal activity of plant extract against *Aspergillus flavus*. Results displayed that all plant extracts significantly (at $p \leq 0.0$) reduced mycelial growth of *A. flavus*. However, at 2000 ppm concentration of plant extracts the mycelial growth was observed in *Delphinium brunonianum* (21.21 mm), *Artemisia brevifolium* (22.15 mm) and *Artemisia absinthium* (22.88 mm) followed by *Uretica dioica* and *Salvia nubicola* compared to control (92.85 mm). (Tables 2 & 3) showed the antifungal efficacy of plant extract against tomato rot fungi *Rhizopus stolonifer* and *Fusarium oxysporum*. At highest ppm concentration of plant extract minimum mycelial growth of *R. stolonifer* and *F. oxysporum* was observed in *Artemisia brevifolium* (20.11 mm; 18.44 mm) compare to control (88.33 mm; 85.33 mm).

**Table 1. Bioefficacy of plant extract at different concentration for growth inhibition (mm) of tomato rotting fungi (*Aspergillus flavus*).**

| Plant extract | $T_1$ (control) | $T_2$ ($400_{ppm}$) | $T_3$ ($800_{ppm}$) | $T_4$ ($1200_{ppm}$) | $T_5$ ($1600_{ppm}$) | $T_6$ ($2000_{ppm}$) |
|---|---|---|---|---|---|---|
| *Salvia nubicola* | $92.85^{A}$ | $72.07^{A}$ | $53.92^{AB}$ | $42.14^{AB}$ | $33.95^{A}$ | $27.03^{A}$ |
| *Uretica dioica* | $92.85^{A}$ | $67.06^{B}$ | $52.62^{AB}$ | $39.92^{AB}$ | $30.52^{B}$ | $23.53^{B}$ |
| *Delphinium brunonianum* | $92.85^{A}$ | $64.38^{BC}$ | $50.63^{B}$ | $39.52^{B}$ | $24.96^{D}$ | $21.21^{B}$ |
| *Artemisia absinthium* | $92.85^{A}$ | $61.81^{C}$ | $49.95^{B}$ | $40.14^{AB}$ | $27.29^{C}$ | $22.88^{B}$ |
| *Artemisia brevifolium* | $92.85^{A}$ | $66.29^{B}$ | $55.55^{A}$ | $42.48^{A}$ | $31.29^{B}$ | $22.15^{B}$ |
| Std.Err.Com | 1.34 | 1.35 | 1.94 | 1.15 | 0.96 | 1.16 |
| CVC | 2.99 | 3.12 | 4.47 | 2.66 | 2.23 | 2.69 |
| Mean | 92.85 | 66.32 | 52.53 | 40.84 | 29.60 | 23.36 |
| SD | 1.39 | 3.73 | 2.842 | 1.73 | 3.44 | 2.37 |
| CV | 1.49 | 5.63 | 5.41 | 4.25 | 11.62 | 10.15 |
| Minimum | 91.44 | 60.66 | 48.46 | 38.45 | 24.44 | 19.88 |
| Maximum | 94.66 | 73.66 | 56.66 | 44.42 | 35.66 | 28.65 |

Means in each row followed by the same letter are not significantly different at LSD test ($p \leq 0.05$)

**Table 2. Bioefficacy of plant extract at different concentration for growth inhibition (mm) of tomato rotting fungi (*Rhizopus stolonifer*).**

| Plant extract | $T_1$ (Control) | $T_2$ ($400_{ppm}$) | $T_3$ ($800_{ppm}$) | $T_4$ ($1200_{ppm}$) | $T_5$ ($1600_{ppm}$) | $T_6$ ($2000_{ppm}$) |
|---|---|---|---|---|---|---|
| *Salvia nubicola* | $88.33^{A}$ | $66.77^{A}$ | $50.89^{A}$ | $39.96^{AB}$ | $31.84^{A}$ | $24.44^{A}$ |
| *Uretica dioica* | $88.33^{A}$ | $65.22^{A}$ | $50.25^{AB}$ | $38.44^{B}$ | $29.10^{A}$ | $22.26^{B}$ |
| *Delphinium brunonianum* | $88.33^{A}$ | $58.18^{B}$ | $50.07^{AB}$ | $40.04^{AB}$ | $23.84^{B}$ | $21.14^{BC}$ |
| *Artemisia absinthium* | $88.33^{A}$ | $60.73^{B}$ | $47.14^{B}$ | $38.84^{AB}$ | $25.73^{B}$ | $20.55^{C}$ |
| *Artemisia brevifolium* | $88.33^{A}$ | $59.77^{B}$ | $50.58^{AB}$ | $40.36^{A}$ | $30.14^{A}$ | $20.11^{C}$ |
| Std.Err.Com | 0.56 | 1.15 | 1.51 | 0.82 | 1.31 | 0.6927 |
| CVC | 1.26 | 2.67 | 3.50 | 1.89 | 3.02 | 1.59 |
| Mean | 88.33 | 62.13 | 49.79 | 39.53 | 28.13 | 21.70 |
| SD | 0.58 | 3.62 | 2.06 | 1.11 | 3.34 | 1.73 |
| CV | 0.66 | 5.83 | 4.14 | 2.81 | 11.87 | 7.98 |
| Min | 87.56 | 55.67 | 46.66 | 37.66 | 22.44 | 19.56 |
| Max | 88.89 | 68.65 | 54.68 | 41.66 | 32.89 | 25.11 |

Means in each row followed by the same letter are not significantly different at LSD test ($p \leq 0.05$).

**Table 3. Bioefficacy of plant extract at different concentration for growth inhibition (mm) of tomato rotting fungi (*Fusarium oxysporum*).**

| Plant Extract | $T_1$ (Control) | $T_2$ ($400_{ppm}$) | $T_3$ ($800_{ppm}$) | $T_4$ ($1200_{ppm}$) | $T_5$ ($1600_{ppm}$) | $T_6$ ($2000_{ppm}$) |
|---|---|---|---|---|---|---|
| *Salvia nubicola* | $85.33^{A}$ | $62.92^{A}$ | $48.56^{A}$ | $38.96^{A}$ | $30.14^{A}$ | $22.44^{A}$ |
| *Uretica dioica* | $85.33^{A}$ | $62.22^{A}$ | $48.253^{A}$ | $37.44^{A}$ | $28.24^{A}$ | $21.26^{AB}$ |
| *Delphinium brunonianum* | $85.33^{A}$ | $57.85^{B}$ | $49.41^{A}$ | $39.37^{A}$ | $22.51^{A}$ | $20.47^{BC}$ |
| *Artemisia absinthium* | $85.33^{A}$ | $57.73^{B}$ | $46.47^{A}$ | $37.50^{A}$ | $25.07^{B}$ | $19.21^{CD}$ |
| *Artemisia brevifolium* | $85.33^{A}$ | $57.66^{B}$ | $48.92^{A}$ | $38.36^{A}$ | $28.81^{B}$ | $18.44^{D}$ |
| Std.Err.Com | 1.28 | 1.63 | 2.21 | 1.0 | 1.14 | 0.72 |
| CVC | 2.86 | 3.77 | 5.11 | 2.30 | 2.63 | 1.67 |
| Mean | 85.33 | 59.67 | 48.32 | 38.33 | 26.95 | 20.36 |
| SD | 1.33 | 2.89 | 2.56 | 1.38 | 3.17 | 1.62 |
| CV | 1.55 | 4.84 | 5.31 | 3.60 | 11.77 | 7.98 |
| Min | 83.56 | 55.67 | 45.44 | 36.11 | 21.44 | 17.66 |
| Max | 86.56 | 64.65 | 54.68 | 40.66 | 30.89 | 23.11 |

Means in each row followed by the same letter are not significantly different at LSD test ($p \leq 0.05$).

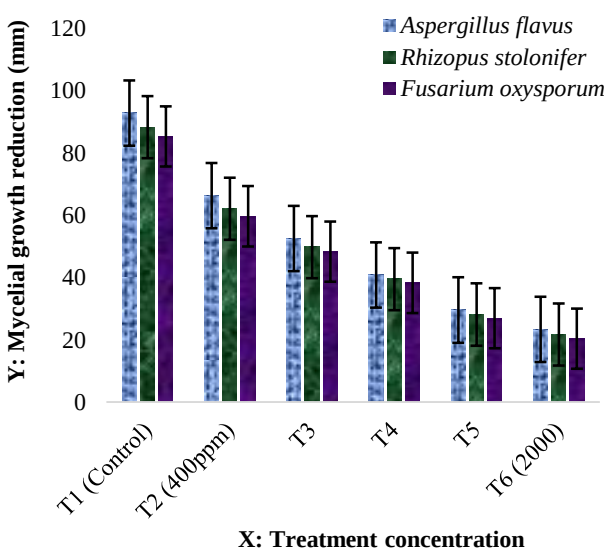


Fig. 2. Mean efficacy treatment concentration against tomato rot fungi.

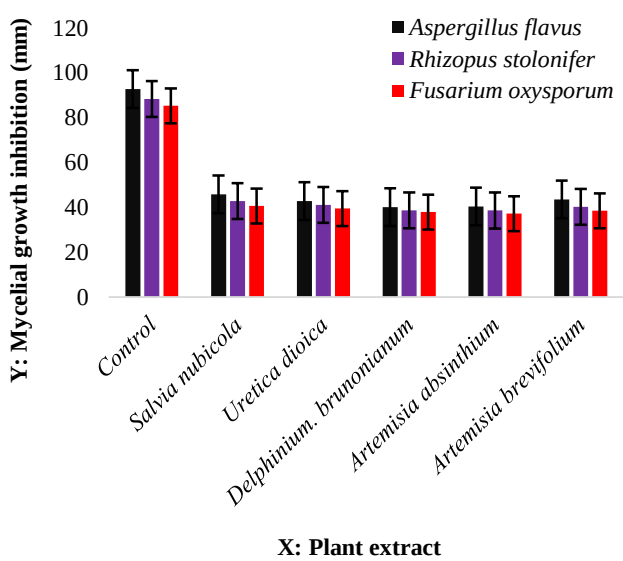


Fig. 3. Mean efficacy of plant extracts against tomato rot fungi.

Overall plant extract concentration and mycelial growth reduction is inversely proportional. Highest mycelial growth reduction was recorded at 2000 ppm concentration while *Artemisia brevifolium* has high antifungal potential to control tomato rotting fungi followed *by Artemisia absinthium* and *Delphinium brunonianum* (Figs. 2-3). The undesirable impact of synthetic chemicals on humans and the environment is a serious concern to researchers. For this intention, substitute methods of reducing chemicals are being developed. One of the alternative and effective methods is to use plant-based biopesticides to control plant pathogenic microorganisms (Plant-derived extracts with antimicrobial activity are being explored all over the World to make easy, cheap and locally produced, particularly for the farmers who cannot afford synthetic pesticides. A number of plants either aromatic, medicinal plants, spices, or others having potential biological properties that can be explored in the field of medical science, pharmacy, cosmetics, and agriculture (Pandey *et al*., 2011). The experimental results of the current study have uncovered significant activity of selected plant extract against the tomato rot fungi. Results revealed highest antifungal potential was found in Artemisia species followed by *Delphinium brunonianum.* The obtained antifungal properties of plant extracts are also in agreement with other researchers including (Baykan *et al*., 2012; Kordali *et al*., 2005). The Artemisia genus belongs to family Asteraceae and approximately ≥ 400 species. *Artemisia* spp has high essential oils contents and it has been used for medicinal purposes (antimalarial, antibacterial, antiviral, nematicidal and fungicidal) for many years [Kirbag *et al*., 2009, Ahameethunisa *et al*., 2012). The main components of this species are camphor, 1, 8-cineole, and chamazulene which act as antifungal properties especially *F. solani* and *F. oxysporum*, *Alternaria* sp. *Aspergillus spp* and *Botrytis cinerea* (Umpierrez *et al*., 2012). Bailen, *et al*., 2013 reported that essential oil extracted from *A. absinthium* showed significant antifungal activity against two rot species i.e *F. oxysporum* and *F. solani* (Bailen *et al*., 2013). *U. dioica* contains phytochmecial including lectins, sterols, terpenes, volatile compounds and fatty acids, polysaccharides, protein, vitamins, minerals and flavonoids. Research has proved that natural sources of compounds with antibacterial, antifungal and antioxidant properties are herbal plants including *Urtica dioica* L. often called common nettle or stinging nettle (Asgarpanah *et al*., 20123: Bisht *et al*., 2012). As an outcome, the plant extracts used in our study was exhibited diverse results of antifungal potential in a dose dependent manner. These plant extracts can be used as biopesticides.

## Conclusion

Tomatoes fungus rots are not only a concern in Pakistan, but also over the world. The plant extract utilized in this study demonstrated good antifungal properties against the most common tomato rot fungus. These plant extracts would be used to enhance the shelf life of tomatoes. These extracts offer a feasible and environmentally safe alternative to treating tomato rot after harvest.

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