FLUORIDE

Quarterly Journal of The International Society for Fluoride Research Inc.

Response of Chili to Different Levels of Fluoride and its Effect on Fusarium Wilt Disease

Unique digital address (Digital object identifier [DOI] equivalent): https://www.fluorideresearch.online/epub/files/372.pdf

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Submitted: 2025 Feb 13
Accepted: 2025 Aug 05
Published as e371: 2025 Aug 09

ABSTRACT

Background: Fluoride is emerging as a new potential environmental pollutant effecting humans, animals and plants.Fusarium wilt, caused by Fusarium oxysporum, is a severe and widespread disease that poses a significant threat to Chilli crops worldwide. This soil-borne pathogen not only reduces yield and quality but also affects the overall health of the plants, leading to substantial economic losses.

Aim: To examine the sodium fluoride's impact on chilli seed germination, growth, and Fusarium wilt severity in a screen house, and to assess the response of Chilli plants to increased fluoride concentrations and Fusarium wilt disease and any other associated risks.

Methodology: Lab tests assessed sodium fluoride's antifungal effect (5-60 ppm) on Fusarium oxysporum and its impact on chilli seed germination and early growth. A screen house experiment then evaluated the effect of the same fluoride concentrations on inoculated chilli plants over 60 days, measuring disease severity and growth parameters (height, biomass, root length) every 15 days.

Findings: The experimental results revealed a complex interplay between sodium fluoride's antifungal properties and its adverse effects on Chilli plant health. In vitro assays showed a dose-dependent inhibition of Fusarium oxysporum f.sp. capsici, with colony diameters decreasing from 6.6 cm at 5 ppm (19.51% inhibition) to 2.3 cm at 60 ppm (71.95% inhibition). Seed germination tests further indicated that higher fluoride concentrations significantly reduced seed viability—from an 80% germination rate at 5 ppm down to 10% at 60 ppm. Additionally, screen house observations highlighted that while sodium fluoride exhibited promising antifungal effects under controlled conditions, its application in growing plants led to increased disease severity (rising from 77.75% at 5 ppm to 94.75% at 60 ppm) and a marked decline in plant growth parameters such as height, biomass, and root length.

Conclusions: This study found that while sodium fluoride effectively inhibits Fusarium oxysporum in lab tests, it worsened Fusarium wilt in chilli plants in screen house trials due to phytotoxicity. This highlights the difficulty of translating in vitro antifungal activity to real-world applications and the need to reduce fluoride concentrations to manage Fusarium wilt without harming crops.

Keywords: *Fluoride, Fusarium oxysporum f.sp capsici, Chili Growth, Seed germination, Disease Severity*

INTRODUCTION

Chilli (*Capsicum annuum* L.) is an important crop grown in tropical and subtropical regions of the world. [1]. In Pakistan, chillies are grown as an important spice crop on the agricultural land of 75000 hectares with an annual production of 119000 tons, which is quite low[2]. Besides, Chillies contain *capsaicin,* an alkaloid which is anti-carcinogenic, anti-mutagenic, antioxidant, and immunosuppressive properties[3,4]. Production of chillies has decreased because of several biotic and

abiotic causes, including fungal diseases [5]. Among fungal diseases, Fusarium wilt caused by *F. oxysporum* f. sp. *capsici* is one of the most damaging diseases worldwide causing losses 15% to 20% in Pakistan's arid agricultural regions [6,7].

Fluoride (F) is a frequently occurring and highly reactive element in the environment and its amount in the Earth's crust is around 0.077% [8] and substances containing Fluoride (F) may leak into the environment through water, soil, and/or the air [9]. Fluoride containing chemicals are widely utilized in all biological industries, and environmental fluoride contamination is common. Fluoride is commonly considered to be one of the three most dangerous air pollutants, along with SO_2 and ozone (O_3) [10]. It might cause health problems in both animals and people while also being phytotoxic to plants. The primary contributors to fluoride pollution of the land, water, and air are the aluminum, fertilizer, ceramic, phosphorus and brick kiln industries. These companies frequently burn coal that releases compounds containing fluoride and Sulphur. Fluoride levels in coal range from 40 to 295 ppm, whereas the quantity of fluoride in clay used to manufacture bricks depends on the size of the brick [11].

Fluoride causes severe harm to sensitive species and is mostly regarded as a phytotoxic contaminant. Fluoride Aqueous solution is absorbed throughout the whole leaf surface [12] and once within the leaf, it travels via the apoplast and respiratory stream before arriving at the margins and apex, where it destroys the leaf when it builds up [13]. Chlorosis is typically the first symptom to appear on leaves due to fluoride and the apical and marginal necrosis that is evident when the exposure length and concentration of this portion raise [14,15].

Phosphate fertilizers, ceramic and brick kiln factories, and volcanic eruptions are the main causes of fluoride in the environment [16,17]. Fluoride has thus been viewed as a growing contaminant that poses risks to regional food security and can hinder agricultural output. Without any human effects, the total soil Fluoride concentration might vary from 20 to 1000 mg/kg [18]. However, in contaminated areas, the soil accessible fluoride level has been observed to be as high as 300 mg 1 kg [19]. High fluoride toxicity to plants is caused by soils with greater amounts of water-soluble and exchangeable F [20]. The complexation of fluoride with Al, Fe, and Ca is what typically transports it through the transpiration stream; however, the pH of the soil affects these complexes' ability to develop [21].

In Pakistan, Peshawar valley is known for production of high quality bricks in brick kilns present in this area. Due to the presence of these large numbers of brick kilns, environmental concerns including elevated levels of Fluoride (F) is on rise. Keeping in view the importance of fluoride as a new emerging pollutant and its effect on plant growth and its synergistic effect to promote plant diseases, the proposed research was carried out in the laboratory as well as in the screen house to assess it toxicity to chilli growth and determine the response of fusarium wilt in excessive fluoride conditions.

MATERIAL AND METHODS

Source of pathogen culture

The Department of Plant Pathology, The University of Agriculture Peshawar culture bank provided the *Fusarium oxysporum* f.sp. *capsici* (FOC) culture. The culture was kept at 4°C. On Potato Dextrose Agar (PDA) Medium, FOC was sub-cultured in an aseptic environment following [22]. The plates were labeled, sealed, and incubated for one to two weeks at 25 °C.

In-vitro antifungal experiment to check the effect of fluoride on Fusarium growth

To check the effect of fluoride on *Fusarium oxysporum* f.sp. *capsici* (FOC), lab experiments were conducted. 20 ml of potato dextrose agar (PDA) media was added to the Petri dishes, and after the media had solidified, 1 ml of sodium fluoride solution from various concentrations was added. The fluoride was added at concentrations of 5, 10, 20, 40, and 60 ppm, with one negative (SDW) and one positive control (fungicide) PDA plates inoculated were with 6 mm plugs of freshly prepared *Fusarium oxysporum* f.sp. *capsici* (FOC) *placed in* the middle of the plate under sterilized conditions. The plates were incubated at 24 to 28°C for 7-14 days. Each treatment was divided into three replications by CRD design. After incubation, the fungal colonies' average diameter was measured [23]. The formula given below was used to calculate the antifungal growth inhibition:

$$I = [(C – T)/C] \times 100$$

Where; I = % inhibition, C = colony diameter in control plate, T = colony diameter in treatment plate.

Germination test of chilli seeds in petri plate with filter paper containing fluoride

Fresh chilli local variety (Tatapuri) seeds were taken and after washing seeds were soaked in a bleach solution (1:1; v/v) for 3 to 4 minutes with continuously shaking [24]. After discarding bleach, seeds were washed with double distilled water three times. Seeds were dried and germinated on sterilized petri plates containing 10 seeds per plate on a filter paper under constant long day light (16-hour light and 8-hour dark) with approximate 50% humidity. Then a concentration of 5, 10, 20, 40 and 60 (ppm) sodium fluoride were added with one control and final volume was 100 ml. Fluoride was applied after plating of seeds and germination was recorded between 7 to 10 days. The experiment has six treatments and each treatment was divided into three replications by CRD design. To check

the germination of chilli seeds the following formula was used [25].

$$\eta \times 100N = G\% \eta \times 100N = G\%$$

n = Number of total germinated seed, N = Total number of seed, G% = Germination percentage

Pots experiment in screen house

Formation of fluoride (NaF) suspension

Fluoride suspension was formed in different concentrations (5mg in one liter distilled water) for 5 ppm, 10 mg in one liter for 10 ppm, 20mg in one liter for 20 ppm, 40mg in one liter for 40 ppm, 60mg in one liter for 60 ppm

Raising of chilli seedlings

Seeds of healthy chilli variety (Tatapuri) were planted in a prepared nursery bed to raise seedlings for transplantation at the screen house of Plant Pathology Department, UAP. The standard horticultural cultural practices were used. In each 15 cm diameter pot with a 1:1:1 mixture of pasteurized sand, clay and farmyard manure, a month-old seedling was transplanted.

Preparation of inoculum

On a PDA media at 27 °C for seven days fresh cultures were inoculated. Using spatula mycelial mats were scratched in order to obtain suspension of spores. The inoculum was suspended in SDW. A concentration of 1×10^4 conidia ml^{-1} spore suspension was adjusted using a haemocytometer.

With the help of a hypodermic syringe 5 cc of inoculum was taken to infect the plants close to the crown area. Inoculum was administered in the morning to enhance the possibility of disease. Normal conventional agronomic practices were performed for raising the plants.

To investigate the effects of fluoride on the Fusarium wilt disease severity and growth parameters of chilli plants, a pot experiment was performed in the screen house of Plant Pathology, at the UAP. Plants were inoculated through a drench method to induce Fusarium wilt disease , T_1 with no inoculation and no fluoride application to healthy control, T_2 having only fluoride treatment 60 ppm without inoculation in solution form with (250 ml each pot), T_3 inoculated control having only inoculation with *Fusarium oxysporum* f.sp *capsici.* Then T4 consisted of inoculation and fluoride with 5 ppm, T5 having 10 ppm fluoride and inoculation, T6 having 20 ppm, T7 40 ppm inoculation and fluoride and T8 60 ppm of fluoride and inoculation. Each treatment was replicated four (4) times under CRD. When the disease's visible symptoms first appeared, the symptoms were compared with the healthy control plants. Plants' height and disease severity were checked on every 15 days' basis.

Statistical Analysis

Statistical software (statistix 8.1) was used to find out the CRD ANOVA on the data taken on different parameters. ANOVA of the data revealed significant, differences among treatments. The least significant differences (LSD) test was applied for means differences. The experimental layout for treatments and seed germination for the conducted experiment is given in (Table.1) and (Table.2) respectively while the layout of the experiment in CRD design is shown in (Table.3).

Table 1. Layout for *in-vitro* experiment

T1	T3	T7	T2	T3	T1	T4
T7	T6	T5	T6	T7	T6	T5
T2	T4	T1	T4	T5	T3	T2

Treatments

T1= sodium fluoride (5 ppm)
T2= sodium fluoride (10 ppm)
T3= sodium fluoride (20 ppm)
T4= sodium fluoride (40 ppm)
T5= sodium fluoride (60 ppm)
T6= positive control (fungicide)
T7= negative control (sterilized distilled water only).

Table 2. Layout for seed germination test

T1	T3	T7	T2	T3	T1
T7	T6	T5	T6	T7	T6
T2	T4	T1	T4	T5	T3

T1= control (Sterilize distilled water)
T2= sodium fluoride (5 ppm)
T3= sodium fluoride (10 ppm)
T4= sodium fluoride (20 ppm)
T5= sodium fluoride (40 ppm)
T6= sodium fluoride (60 ppm)

Table 3. Table of treatments randomization in CRD for the screen house experiment.

T1	T3	T7	T2	T3	T1	T4	T1
T7	T6	T5	T6	T7	T6	T5	T7
T2	T4	T1	T4	T5	T3	T2	T2
T1	T3	T7	T2	T3	T1	T4	T1

T1 = Healthy control
T2 = Only Fluoride (60 ppm)
T3 = Inoculation (control)
T4 = Fluoride and inoculation (5 ppm)
T5 = Fluoride and inoculation (10 ppm)
T6 = Fluoride and inoculation (20 ppm)
T7 = Fluoride and inoculation (40 ppm)
T8 = Fluoride and inoculation (60 ppm)

Data recording

Data was recorded on the following parameters: disease severity, plant height, root length, fresh biomass, and dry weight at harvest. A scale 0 to 4 was used to the rate of Fusarium wilt by [26]. (Table 4) provides a quantitative assessment of Fusarium wilt disease severity in chili peppers.

Table 4. Scale of disease severity for Fusarium wilt of chilli

0	*0% infection*
1	*A few leaves turn yellow in around 25% of cases with mild infection.*
2	*Moderate infection (50% leaves turn into wilted and two or three leaves become yellow).*
3	*Extensive infection (75% leaves become wilted and growth is reduced and all leaves become yellow).*
4	*Complete infection (100% leaves become wilted, plants die and the whole plant leaves become yellow).*

Using disease severity (%) formula (DS = (T/N) × 100) the data was transformed to percentage disease severity. Where T represents the infected leaves per plant while N indicates the total number of plants [26].

RESULTS

In-vitro antifungal activity of sodium fluoride on growth of F. oxysporum f. sp. capsici

Different concentrations of sodium fluoride were tested against fungus F. oxysporum f.sp. capsici using PDA medium as shown in (Fig.A,Fig. B and Fig. C) . At a concentration of sodium fluoride 5, 10, 20, 40, 60 ppm significant antifungal action was noted. Each treatment showed an impact on fungus development and greatly differed from the others treatment in terms of antifungal activity (Table 5). The antifungal activity of sodium fluorides is generally enhanced with increased concentration. After 7 days of incubation, the smallest colony diameter of the fungus (2.3 cm) was observed in plates where 60 ppm was applied achieving highest growth inhibition 71.95% followed by (2.8 cm) with 40 ppm, 65.85% growth inhibition. At 5 ppm maximum colony diameter (6.6 cm) was observed, smallest 19.51 % fungal growth inhibition was found while at 10 ppm, 4.8 cm colony diameter was observed inhibiting 41.46% fungal growth and at 20 ppm, 3.8 cm colony diameter was observed, inhibiting 53.65% fungal growth. The negative control (sterilized distilled water) was least effective allowing large colony diameter (8.2 cm) and positive control Difenoconazole showed over all smallest colony diameter (1.86 cm) which inhibiting 77.31 % fungal growth.

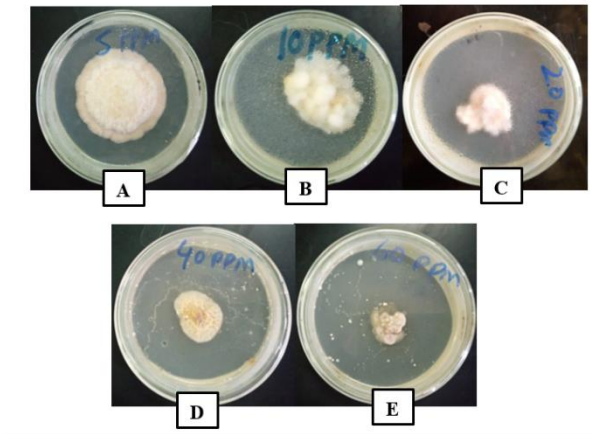

Figure 1. Efficiency of different concentrations of sodium fluoride on growth of F.oxysporum f. sp. capsici. A = 5 ppm fluoride, B =10 ppm fluoride, C =20 ppm fluoride, D =40 ppm fluoride, and E = 60 ppm fluoride

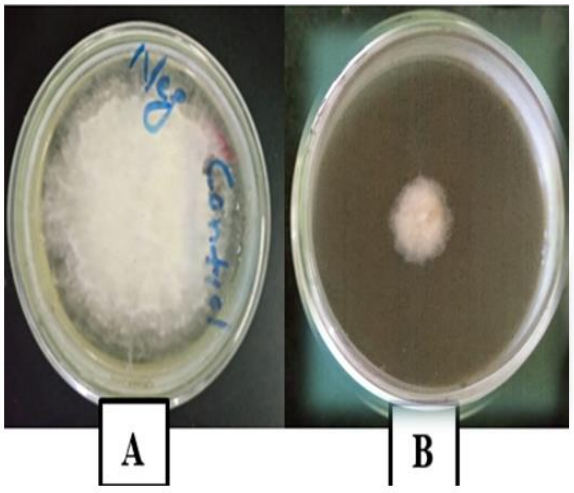

Figure 2. Control effect on the in-vitro growth of Fusarium oxysporum f. sp. capsici grown on PDA medium. (A) Maximum growth was observed on negative control (sterilized water). (B) Minimum growth was observed on positive control fungicide (Difenoconazole)

Chilli Seed Germination Test (%)

Analysis of data showed that different concentrations of sodium fluoride gave significant differences regarding seed germination percentage. Increase in concentration of the sodium fluoride up to 60 ppm decreased the seed germination percentage.

Table 5. Effect of different concentration of fluoride on colony growth of *F. oxysporum* f.sp *capsici*.

Treatment	Colony diameter (cm)	Reduction in colony growth (%)
5 ppm	6.6 b	19.51
10 ppm	4.8 c	41.46
20 ppm	3.8 d	53.65
40 ppm	2.8 e	65.85
60 ppm	2.3 f	71.95
Difenoconazole	1.86 g	77.31
Negative control (SDW)	8.2 a	
LSD 0.05	0.33	

Mean followed by different letters differ significantly from each other at 5% level of probability.

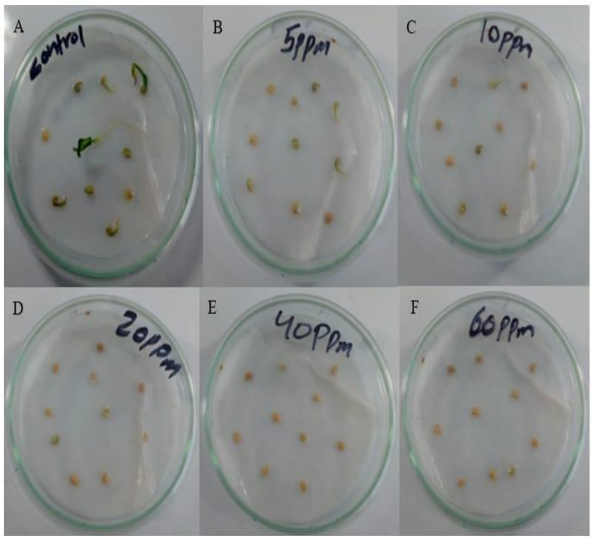

Figure 4. Effect of different concentrations of sodium fluoride on germination (%) of chilli seeds. A = control (90% seed germination) B = 5 ppm fluoride, C =10 ppm fluoride, D=20 ppm, E=40 ppm, fluoride and F= 60 ppm fluoride (10% germination).

In the control treatment (T1) maximum (90%) seed germination was observed followed by 80% recorded for 5 ppm sodium fluoride application (T_2) and 76% recorded for 10 ppm sodium fluoride application (T_3). The seed germination percentage decreased as the concentration of sodium fluoride increased. Similarly, the seed germination percentage of 20 ppm sodium fluoride application (T_4) was 60% and 40 ppm sodium fluoride application (T_5) was (30%) as shown in (Table 6). The minimum seed germination percentage 10% was recorded for 60 ppm (T_6) sodium fluoride application. The treatment T2 (5 ppm) 80% and T3 (10 ppm) 76% statistically non-significant to each other.

Among the treatments, the control showed maximum seed germination percentage (90 %) while the treatment (T_6) sodium fluoride application showed minimum (10 %) seed germination percentage.

Table 6. Effect of different concentration of fluoride on chilli Seed germination percentage

Treatments	Fluoride Concentrations (ppm)	Seeds germination %
T1	0	90 a
T2	5	80 b
T3	10	76 b
T4	20	60 c
T5	40	30 d
T6	60	10 e
$LSD_{0.05}$		8.3126

Mean followed by different letters differ significantly from each other at 5% level of probability.

Screen house experiment

Effect of sodium fluoride on disease severity (%)

Data on disease severity was taken four times, at 15, 30, 45, and 60 days after inoculation of plants with *Fusarium. oxysporum* f. sp. *capsici*. The findings are presented in (Table 7). Increasing the quantity of sodium fluoride was often associated with an increase in disease severity. (T1) Healthy control and (T2) fluoride treatment without inoculation showed no disease severity (0) after 15 days. In chilli plants that were planted in soil drenched with 60 ppm (T8) sodium fluoride with inoculation, disease severity steadily increased as the amount of fluoride was raised and reached 42%. T3 (inoculated) control recorded lowest disease 25.25%, while the application of sodium fluoride at the rate of (T4) 5 ppm, (T5) 10 ppm, (T6) 20 ppm and (T7) 40 ppm showed 26%, 29%, 33%, and 36.50 % disease severity respectively. The same trend was observed for disease severity recordings at 30, 45 and 60 days of inoculation.

Table 7. Effect of disease severity on chilli plants treated with different concentrations of (NaF).

Treatments	15 Day	30 Day	45 Day	60 Day
Healthy	0 f	0 f	0 f	0 f
Only fluoride 60 ppm	0 f	0 f	0 f	0 f
inoculated control	25.25 e	51 e	65.25 e	77.75 e
5 ppm	26 e	51.75 e	67.25 e	80 e
10 ppm	29 d	55 d	70 d	83 d
20 ppm	33 c	58.50 c	74 c	85.75 c
40 ppm	36 b	63 b	77.75 b	89.25 b
60 ppm	42 a	68 a	82.75 a	94.75 a
$LSD_{0.05}$	1.87	3.20	2.45	2.54

Means followed by different letters differ significantly from each other at 5% level of probability.

Effect of sodium fluoride on chilli plant growth

When sodium fluoride was applied to soil that has been artificially inoculated with *F. oxysporum* f. sp. *capsici* the impacts on growth characteristics of chilli plants (plant height, fresh biomass, dry biomass and root length) were analysis. Data demonstrated that varying sodium fluoride doses resulted in considerably variable plant growth characteristics. Increase in dose of the sodium fluoride up to 60 ppm with inoculation decreased the plant growth as shown in (Table 8).

Plant height (cm)

The increase in the concentrations of NaF resulted in the decrease of height of chilli plants. On an average, the highest dose i.e. T1 the healthy control treatment showed the maximum height (73.25 cm) in chilli plants, T2 fluoride without pathogen inoculation showed (26.87 cm) cm plant height, while T8 60 ppm with pathogen inoculation showed lowest plant height (17.37 cm). The T3 inoculated control showed the highest height after healthy control (42.50 cm). chilli height decreased with higher rate in the remaining treatments showing T4 (41) cm, T5 (37.50 cm), T6 (33.25 cm) and T7 (28.20 cm) by 5 ppm, 10 ppm, 20 ppm and 40 ppm sodium fluoride concentration. Among the treatments, the application of healthy control treatment (showed maximum plant height (73.25 cm) and the treatment T_8 sodium fluoride application showed minimum (17.37 cm g) height. as shown in (Table 8).

Table 8. Effect of different concentration of Fluoride on growth parameters of chilli plants inoculated with *Fusarium oxysporum* f.sp. *capsici.*

Treatments	Plant height (cm)	Fresh biomass (g)	Dry biomass (g)	Root length (cm)
Healthy control	73.25 a	32.32 a	7.25 a	19.20 a
Only fluoride 60 ppm	26.87 e	3.4 e	1.57 e	3.45 e
Inoculated control	42.50 b	10.97 b	3.5 b	13.20 b
5 ppm	41 b	10.50 b	3..25 b	12.87 b
10 ppm	37.50 c	7.20 c	2.50 c	9.9 c
20 ppm	33.25 d	5.40 d	1.95 d	6.3 d
40 ppm	28.20 e	3.87e	1.62 de	3.9 e
60 ppm	17.37 f	2.5 f	0.6 f	1.9 f
$LSD_{0.05}$	1.83	0.61	0.34	0.49

Mean followed by different letters differ significantly from each other at 5% level of probability.

Fresh Biomass (g)

Data showed that different concentrations of sodium fluoride gave significant differences in terms of plant fresh biomass. Increase in concentration of the sodium fluoride up to 60 ppm with inoculation decreased the fresh biomass of chilli plants.

The maximum fresh biomass of chilli plants (32.32 g) was recorded in the healthy control plants treatment T_1 followed by T2 fluoride treatment 60 ppm without inoculation shows (3.40 g). T_3 inoculated control recorded, (10.97 g). (10.50 g) by the 5 ppm sodium fluoride application T_4 and (7.2 g) recorded for 10 ppm sodium fluoride application T_5. The fresh biomass decreased as the doses of (NaF) increased. Similarly, the fresh biomass of 20 ppm sodium fluoride application T_6 showed the (5.4 g) and 40 ppm sodium fluoride applications T_7 showed the (3.87 g). The minimum fresh biomass (2.5 g) was recorded for 60 ppm (T_8) sodium fluoride application.

Among the treatments, the application of healthy control treatment (showed maximum fresh biomass

(32.32 g) and the treatment T_8 sodium fluoride application showed minimum (2.5 g) fresh biomass. As shown in (Table 8).

Dry weight (g)

Data revealed that the dry weight of the plants greatly varied depending on the sodium fluoride doses. Increase in dose of the sodium fluoride up to 60 ppm with inoculation decreased the plant dry weight.

The maximum dry weight of chilli plants (7.2 g) was recorded in the healthy control T_1, T2 60 ppm fluoride without inoculation show (1.57 g) dry weight, followed by (3.5 g) recoded for inoculation control (T_3), (3.25 g) by the 5 ppm sodium fluoride T_4 and (2.5 g) recoded for 10 ppm sodium fluoride application T_5. The dry weight decreased as the concentration of sodium fluoride increased. Similarly, the dry weight of T_6 20 ppm sodium fluoride application showed (1.95 g) and T_7 40 ppm sodium fluoride application show (1.62 g) sodium fluoride concentration as shown in (Table 8) after that showed the minimum dry weight (0.6 g) was recorded for 60 ppm (T_8) sodium fluoride application.

Among the treatments, the application of healthy control (showed maximum dry weight (7.2 g) while the treatment (T_8) sodium fluoride application showed minimum (0.6 g) dry weight.

Root Length (cm)

Data showed that different concentrations of sodium fluoride showed significant differences regarding plant root lengths with increase in concentration of the sodium fluoride up to 60 ppm with inoculation decreased the root length of chilli plants.

The maximum root length of chilli plants (19.20 cm) was recorded in the healthy control. T_1. Fluoride 60 ppm without inoculation T2 show (3.45 cm) followed by (13.20 cm) for inoculation control T_3, the (12.87 cm) by the 5 ppm sodium fluoride application T_4 and (9.9 cm) for 10 ppm sodium fluoride application (T_5). The root length was decreased as the concentration of sodium fluoride increased. Similarly, the root length of 20 ppm sodium fluoride application (T_6) showed the (6.3 cm) and 40 ppm sodium fluoride applications T_7 showed the (3.9 cm). The minimum root length (1.9 cm) was for 60 ppm (T_8) sodium fluoride application.as shown in (Table 8).

Among the treatments, the application of healthy control (showed maximum root length (19.12 cm) while the treatment T_8 sodium fluoride application with inoculation showed minimum (1.9 cm) root length.

The second data was gathered 30 days following the inoculation. The combination of inoculation with (T8) 60 ppm sodium fluoride treatment resulted in the greatest disease severity, 68%. By increasing the sodium fluoride, the disease severity gradually increased. The (T3) inoculated control recorded the lowest 51% disease severity. The application of sodium fluoride at the rate of (T4) 5 ppm, (T5) 10 ppm, (T6) 20 ppm and (T7) 40 ppm showed 51.75%, 55%, 58.50% and 63% disease severity, respectively. While Healthy control and fluoride treatment without inoculation showed no disease severity (0%)

After 45 days of inoculations the third set of data was taken, a clearly significant difference was observed between the treatments in terms of the progression of the disease on chilli plants. The highest dose i.e. (T8) 60 ppm with inoculation allowed maximum disease severity (82.75%). And (T3) inoculated control record 65.25% minimum disease, while healthy control and fluoride treatment without inoculation showed (0%) disease severity while in the remaining treatments the disease severity increased with higher rate showing 67.25%, 70.25%, 74% and 77.75% by (T3) 5 ppm, (T4) 10 ppm, (T5) 20 ppm and (T6) 40 ppm sodium fluoride.

Fourth data were recorded after 60 days of inoculation. The maximum disease severity i.e. 94.75% was noted with (T8) 60 ppm sodium fluoride application with inoculation and (T3) inoculated control record lowest disease 77.75%. The gradual increase in disease severity was observed by increasing the sodium fluoride. The application of sodium fluoride at the rate of (T4) 5 ppm, (T5 10) ppm, (T6 20) ppm and (T7 40) ppm showed 80%, 83%, 85.75% and 89.25% disease severity respectively, the treatment T3 inoculated control 77.75% and T4 5 ppm 80 % are statistically non-significant with each other, while Healthy control and fluoride treatment without inoculation recorded no disease severity (0%).

The disease severity data results showed that the highest treatment dose of sodium fluoride showed maximum disease severity value as compared to inoculated control.

DISCUSSION

Fluoride is 13th most abundant element of the earth crust and represents about 0.3 g/kg of earth's crust, The major sources of fluoride are natural include mineral rock (fluorspar, phosphate, cryolite, apatite and mica), in mountainous regions sediments of marine origin, gneissic and granitic rocks and volcanic rocks [31]. Fluoride is an emerging toxic substance which causes toxicity to plants through different mechanisms by air in the form of HF, in soil by disturbing soil ecology and contaminating the groundwater by dissolving through rocks. In the environment, fluoride is released mainly from industries, prostatic fertilizers, tiles, ceramics, bricks and other human activity. These sources contaminate water, soil, air and adversely affect plants growth and productivity [32]. Recently, Fluoride (F) contamination has increased in Peshawar valley due to excessive coal burning by brick kilns posing a significant threat to humans, animals as well as plants and inducing new plant diseases.

Fusarium wilt caused by *F. oxysporum* f. sp. *capsici* is a cosmopolitan soil-borne pathogen affecting many important crops including chillies[27]. The pathogen causes huge losses in many crops including Chillies [7]. Different management strategies are used to manage Fusarium wilt diseases, but due to the presence of pathogen in the soil, management of Fusarium wilt diseases is still a challenge worldwide [28,29,30]. Emerging of new pollutants like Fluoride may aggravate the already existing problem of Fusarium wilt management.

In our studies, different concentrations of sodium fluoride were tested in *in-vitro* conditions for their interaction with *Fusarium oxysporum* f. sp. *capsici.* The growth of FOC was adversely affected by different sodium fluoride concentrations under *in-vitro* conditions. The colony diameter of *F. oxysporum* f. sp. *capsici* was most significantly reduced when increased concentration of fluoride (60 ppm) was used. The treatment using 5 ppm (NaF) caused minimum reduction in the colony diameter of *Fusarium oxysporum*. Sodium fluoride is an efficient fungi static agent and is able to inhibit mycelia growth of vascular fungus [33]. The antifungal properties of sodium fluoride may be responsible for its ability to suppress fungal growth. In comparison to lower concentrations, larger concentrations of sodium fluoride naturally include higher levels of the bioactive chemicals that are anti-microbial, which results in increased fungal suppression. There were significant differences in the inhibitory effects of sodium salts particularly on fungus mycelial development, as demonstrated by the fact that sodium meta bisulfite and sodium fluoride at p (0.05) and 2% (w/v) concentrations completely inhibited fungus mycelial development while other salts did not stopped it [23].

Results from Screen house experiments showed that higher sodium fluoride concentration (60 ppm) with pathogen inoculation in soil, resulted in highest disease severity recorded, and the treatment 5 ppm concentration recorded lowest disease severity with the increase in concentration of sodium fluoride, disease severity increases and reduction occurs in growth of plant (height, fresh biomass, and dry biomass). Both sodium fluoride and *Fusarium oxysporum* f. sp. *capsici* increased disease severity and decreased growth parameters of the Chilli plants. The Treatment (T2) 60 ppm when applied without pathogen inoculation plants exhibited minimum plant growth (Fresh biomass, Plant height, and Dry weight and Root length) as compared to other treatments indicating that higher levels of Fluoride (F) are detrimental to plant growth. Similar results by other researchers are reported in chilli, cotton and Maize crops, an increase in fluoride concentration decreased net primary productivity (NPP). All plant metrics were drastically lowered by all degrees of F treatments [33].

In the seed germination test, data showed that increased concentration of the sodium fluoride up to 60 ppm decreased the seed germination percentage. Among the treatments, the application of 5 ppm (showed maximum seed germination percentage (80%) while the treatment (T_6) sodium fluoride application showed minimum (10%) seed germination percentage. Previously, in two tomato varieties it was found that increase in fluoride concentration (10, 25, 50, and 100 ppm) adversely affected seed germination rate [34]. Similar findings were reported by [35] in their *Triticum aestivum* var research, which showed that sodium fluoride reduced seed germination from 100% after 7 days of treatment with control to 88% after 20 mg NaF/L. [36] provided evidence that higher fluoride uptake and associated impairment in nutrient and trace metal accumulation caused leaf injury accompanied by powdery mildew infestation in wheat.

Our result showed that *in-vitro* experiment, inorganic sodium fluoride inhibited mycelium growth of *F. oxysporum* f. sp. *capsici.* But in a screen house experiment, fluoride toxicity on plants *and Fusarium oxysporum* results showed that combined effect of fluoride with inoculum disease severity increased. Our results suggest that higher concentrations of Fluoride (F) decrease plant growth and increase Fusarium wilt disease severity possibly by putting the plants under physiological stress.

CONCLUSION

Sodium fluoride increased the disease severity of fusarium wilt pathogen and also reduced plant height, biomass, dry weight, root length, of the chilli plant. Sodium fluoride applied 60 ppm to soil accompanied with *Fusarium oxysporum* f. sp. *capsici* was noted as more toxic than other concentrations. In order to minimize the yield losses in crops grown in Fluoride contaminated soils, crop varieties withstanding high concentrations of fluoride may be grown. Additionally, measures should be taken to check the release of Fluoride (F) to contaminate the environment.

ACKNOWLEDGEMENTS

Authors are thankful to the Departments of Plant Pathology and Agricultural Chemistry and Biochemistry, The University of Agriculture, Peshawar for providing space and consumables to carry out the experiments.

AUTHOR'S CONTRIBUTION

The study was conducted by Hamid Munir under the supervision of Dr. Syed Sartaj Alam. Muhammad Zakir, Waleed Nazim and Sara Aslam helped in the conduction

of Lab and screenhouse experiments . The manuscript is written by Dr Syed Sartaj Alam.

FUNDING

"Not applicable".

CONFLICT OF INTERESTS

"None".

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