# Evaluation of Some Plant Defense Inducing Chemicals for Their Potential to Induce Faba bean (Vicia Faba L.) Disease Resistance against its Major Diseases

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**Abstract:** Faba bean (Vicia faba L.) is a globally grown legume crop. Currently, Ethiopian is the second largest faba bean producing country after China. Faba bean production in Ethiopia is highly impacted by major fungal disease Chocolate spot (Botrytis fabae Sardina). The field experiment was done at Sinana Agricultural Research Center research site in RCBD with three replications for three years. The intention was to evaluate effect of eight (8) signaling chemicals (Sodium Carbonate ($NaCO_3$), Di-Potassium hydrogen phosphate ($K_2HPO_4$), Calcium chloride ($CaCl_2$), Ascorbic Acid ($C_6H_8O_6$), Salicylic Acid ($C_7H_6O_3$), Boric Acid ($H_3BO_3$), Citric Acid ($C_6H_8O_7$), and Oxalic Acid ($C_2H_2O_4$)) on development of faba bean diseases. The signaling chemicals were as seed treatment, as foliar spray and as seed treatment and foliar. Spray of a fungicide Mancozeb 80% WP at a rate of 2.5 kg/ha and untreated plot was included as positive and negative controls, respectively. ANOVA has shown significant difference ($P \leq 0.05$) between treatments for faba bean diseases severity. Seed treatment of the chemicals has reduced diseases than the foliar spray. Some of the chemicals (Salicylic acid foliar spray, seed treatment and foliar spray of Boric acid, seed treatment and foliar spray of Calcium chloride, Ascorbic acid foliar spray and Di-Potassium hydrogen phosphate ($K_2HPO_4$) applied as foliar spray) have favored the development of faba bean diseases and the highest chocolate spot severity (32.1%) was recorded for plots treated by these chemicals. Additionally, salicylic acid has inhibited the germination of seeds significantly. The lowest chocolate spot severity (22.2%) was recorded from calcium chloride seed treated plot. Similarly, the applications of signaling chemicals have significantly influenced the faba bean agronomic performance. The high biomass yield of 1383.3 kg/ha was obtained from plots treated with Calcium chloride as seed treatment. The highest TKW (515.2g) and the lowest TKW (466.2g) was recorded from plot treated by Sodium Carbonate ($NaCO_3$) as seed treatment and Ascorbic Acid ($C_6H_8O_6$) applied as foliar treatment, respectively. Regarding grain yield, the highest (4008.1 kg/ha) was recorded from plot treated by calcium chloride seed treatment and the lowest (0.6 kg/ha) was recorded from salicylic acid seed treated plot. From the result of this study, it is observed that seed treatment of calcium chloride has improved the yield of faba bean. Therefore, the seed treatment of calcium chloride at a concentration of 20mM is recommended for the improvement of faba bean productivity.

**Keywords:** Faba bean, Chocolate spot, Botrytis fabae Sardina, Signaling chemicals, ANOVA

## Introduction

Chocolate spot caused by *Botrytis fabae* (Sardina) is the most important disease of faba bean all over the world (Faried *et al.,* 2013). It is considered one of the most economically important diseases that damage the foliage, limit photosynthesis activity, and reduce faba bean production globally (Torres *et al*., 2004). Currently, controlling this disease depends mainly on fungicidal treatments. However, the environmental pollution caused by excessive use and misuse of agrochemicals, has led to considerable changes in people's attitudes towards the use of synthetic pesticides in agriculture (Faried *et al.,* 2013). Many investigations concerning the use of abiotic factors for induction of plant resistance against several diseases have been undertaken. Potassium salts ($K_2HPO_4$ or $KNO_3$) as a chemical agent for induction of plant resistance had great attention in many of these reports (Marin *et al*., 2002). There has been considerable interest in the use of potassium bicarbonate ($KHCO_3$) for controlling various fungal diseases in plants (Smilanick *et al*., 2006). Bicarbonates are also widely used in the food industry and were found to suppress several fungal plant diseases (Ziv and Zitter, 1992). Spraying with $KHCO_3$ solution provided the most effective protection against plant diseases (Abd-El-Kareem, 2007). On Faba bean chocolate spot; these bicarbonates has showed as high as 74% disease reduction on the field condition which is accompanied by as high as more than 65% yield increase and 100 % reduction in the linear growth of *Botrytis fabae* in the laboratory (Faried *et al.,* 2013). On the other hand, induction of resistance using phosphates was reported in many plants (Abd-El-Kareem *et al.,* 2004).

Using phosphates, led to a chocolate spot disease reduction of more than 71% which has resulted to more than 58% yield increment (Faried *et al.,* 2013). Therefore, the current trend in crop protection against diseases is to apply different chemical inducers that will stimulate the inherent defense mechanisms of the host plant. Such chemical inducers are assumed to be much more environmentally safe than synthetic fungicides, to have a lower economic cost of production for farmers and to create induced systematic resistance in the hosts against several pathogens.

## Materials and Methods

### Description of experimental sites

The field experiment was conducted during main cropping season "Bona" of the Bale area at Sinana Agricultural Research Center (SARC) on-station trial field. It was conducted since 2014/15 for 3 years. SARC is located at 463 km far from the central city Addis Ababa to the south-east. Its geographic location is 07° 07' N latitude and 40° 10'E longitude with an elevation of 2400 masl. The area receives 750–1000 mm mean annual rain fall and have mean annual temperature of 9–21 °C (Nefo *et al.,* 2008).

### Treatments and design

Randomized Complete Block Design with 3 replications was used to layout the experiment. A Faba bean variety 'Mosisa' was used as test crop for the treatments. Eight signaling chemicals were evaluated at a rate of 20mM concentration. A Mancozeb (*Dithane M-45)* 80% WP fungicide was sprayed at 2.5kg/ha as positive control (standard check) and a plot with no fungicide or signaling chemicals treatments was included as negative control (local check) for treatments comparison. The application of signaling chemicals were arranged in three ways: (1) seed treatment; Faba bean seeds were soaked in 1L of 20mM concentration signaling chemicals for 24 hours before planting, (2) seed treatment and foliar spray of signaling chemicals with 1L of 20mM and 500 ml of 20mM concentration signaling chemicals, respectively at the beginning of the disease infection, and (3) foliar spray of signaling chemicals with 500 ml of 20mM concentration (Table 1). The plot size was 1.6m x 3m which contains 4 seeding rows. Between row, plot and block spacing was 0.4m, 1m and 1.5m, respectively. The seed rate was 125kg/ha and the fertilizer rate was 100 kg NPS/ha. The disease development was rated using 1-9 scoring scale (Bernier *et al.,* 1993).

**Table1: Treatments arrangement**

| Treatment No. | Chemical inducers | Application form |
|---|---|---|
| 1 | Sodium Carbonate ($NaCO_3$) | Seed treatment |
| 2 | Di-Potassium hydrogen phosphate ($K_2HPO_4$) | Seed treatment |
| 3 | Calcium chloride ($CaCl_2$) | Seed treatment |
| 4 | Ascorbic Acid ($C_6H_8O_6$) | Seed treatment |
| 5 | Salicylic Acid ($C_7H_6O_3$) | Seed treatment |
| 6 | Boric Acid ($H_3BO_3$) | Seed treatment |
| 7 | Citric Acid ($C_6H_8O_7$) | Seed treatment |
| 8 | Oxalic Acid ($C_2H_2O_4$) | Seed treatment |
| 9 | Sodium Carbonate ($NaCO_3$) | Foliar spray |
| 10 | Di-Potassium hydrogen phosphate ($K_2HPO_4$) | Foliar spray |
| 11 | Calcium chloride ($Ca Cl_2$) | Foliar spray |
| 12 | Ascorbic Acid ($C_6H_8O_6$) | Foliar spray |
| 13 | Salicylic Acid ($C_7H_6O_3$) | Foliar spray |
| 14 | Boric Acid ($H_3BO_3$) | Foliar spray |
| 15 | Citric Acid ($C_6H_8O_7$) | Foliar spray |
| 16 | Oxalic Acid ($C_2H_2O_4$) | Foliar spray |
| 17 | Sodium Carbonate ($NaCO_3$) | Seed treatment & Foliar spray |
| 18 | Di-Potassium hydrogen phosphate ($K_2HPO_4$) | Seed treatment & Foliar spray |
| 19 | Calcium chloride ($Ca Cl_2$) | Seed treatment & Foliar spray |
| 20 | Ascorbic Acid ($C_6H_8O_6$) | Seed treatment & Foliar spray |

| | | |
|---|---|---|
| 21 | Salicylic Acid ($C_7H_6O_3$) | Seed treatment & Foliar spray |
| 22 | Boric Acid ($H_3BO_3$) | Seed treatment & Foliar spray |
| 23 | Citric Acid ($C_6H_8O_7$) | Seed treatment & Foliar spray |
| 24 | Oxalic Acid ($C_2H_2O_4$) | Seed treatment & Foliar spray |
| 25 | Positive control (Mancozeb 80% WP) | Mancozeb spray @ 2.5 kg/ha |
| 26 | Negative control (Untreated control) | No spray |

**Statistical analysis**

Logistic, [ln [(Y/1-Y)] model was used for estimation of disease parameters (Vander Plank, 1963). Therefore, experimental data under different treatments were analyzed using logistic model, ln[y/(1-y)] with the SAS Procedure (SAS Institute, 1998). Diseases were scored based on 1-9 scoring scale, where 1= No disease symptoms or very small specks; 3= few small discrete lesions; 5= some coalesced lesions with some defoliation; 7= large coalesced sporulating lesions, 50% defoliation and some dead plant; and 9= Extensive lesions on leaves, stems and pods, severe defoliation, heavy sporulation, stem girdling, blackening and death of more than 80% of plants (Bernier *et al.,* 1993). The collected diseases data on the basis of scale were further converted in to percentage severity index (PSI) (Wheeler (1969). List significant difference (LSD) technique at 95% confidence interval was used for mean separation. Area Under Disease Progress Curve (AUDPC) and apparent infection rate (r) were calculated for each treatment (Shaner and Finney, 1977). ANOVA was performed for PSI, AUDPC and r using SAS version 9.1.3. The association between disease parameters with yield and yield-related traits was assessed using Correlation and Regression analyses.

$$\text{PSI} = \frac{\text{Sum of Numerical Ratings X 100}}{\text{Number of Plants Scored X Maximum Score on Scale}} \ldots\ldots\ldots\ldots 1 \text{ (Wheeler, 1969)}$$

**Results and Discussion**

Different signaling chemicals were evaluated for their potential to induce systemic acquired resistance (SAR) against Faba bean diseases. Some of the evaluated signaling chemicals were found to be effective. The result recorded was comparable with the standard check fungicide Mancozeb 80% WP (Table 2, Figure 1 A, B and C). Analysis of variance (ANOVA) has shown significant difference *($P \leq 0.05$)* between treatments for chocolate spot, rust and Ascochyta blight diseases severity (Table 2). In general terms, the application of the signaling chemicals has reduced the severity of diseases. Mansour *et al.,* (2021), have found and published that the treatment of signaling chemicals has checked the growth and development of faba bean diseases. The highest chocolate spot disease severity of 32.1% were scored from plots which have received Salicylic acid foliar spray, seed treatment and foliar spray of Boric acid, seed treatment and foliar spray of Calcium chloride, Ascorbic acid foliar spray and Di-Potassium hydrogen phosphate ($K_2HPO_4$) applied as foliar spray (Table 2). An application of Salicylic acid in the form of seed treatment (soaking of seeds in 20mM Salicylic acid for 24 hours) has significantly inhibited the growth of the faba bean seedling and promoted the severity of faba bean diseases severity. This result is in agreement with the findings of Lv *et al.,* (2020). They reported that treatment of faba bean seed by salicylic acid has significantly inhibited seedling growth and promoted fusarium wilt disease of faba bean. The lowest chocolate spot severity (22.2%) was recorded from plot treated with calcium chloride as Seed treatment (Table 2). The influence of signaling chemicals on growth and development of faba bean diseases is well studied. Some signaling chemicals have found to retard the germination of *Uromyces viciae-fabae* urediospore (Abada *et al.,* 2019). Similarly, an application of signaling chemicals affects the germination and growth of *Botrytis fabae* Sard. spores (Elwakil *et al.,* 2016). In the current study, seed treatments of the signaling chemicals have reduced the diseases severity compared to the foliar application Abada *et al.,* (2019) have also reported similar result from their study on faba bean rust (*Uromyces viciae-fabae*).

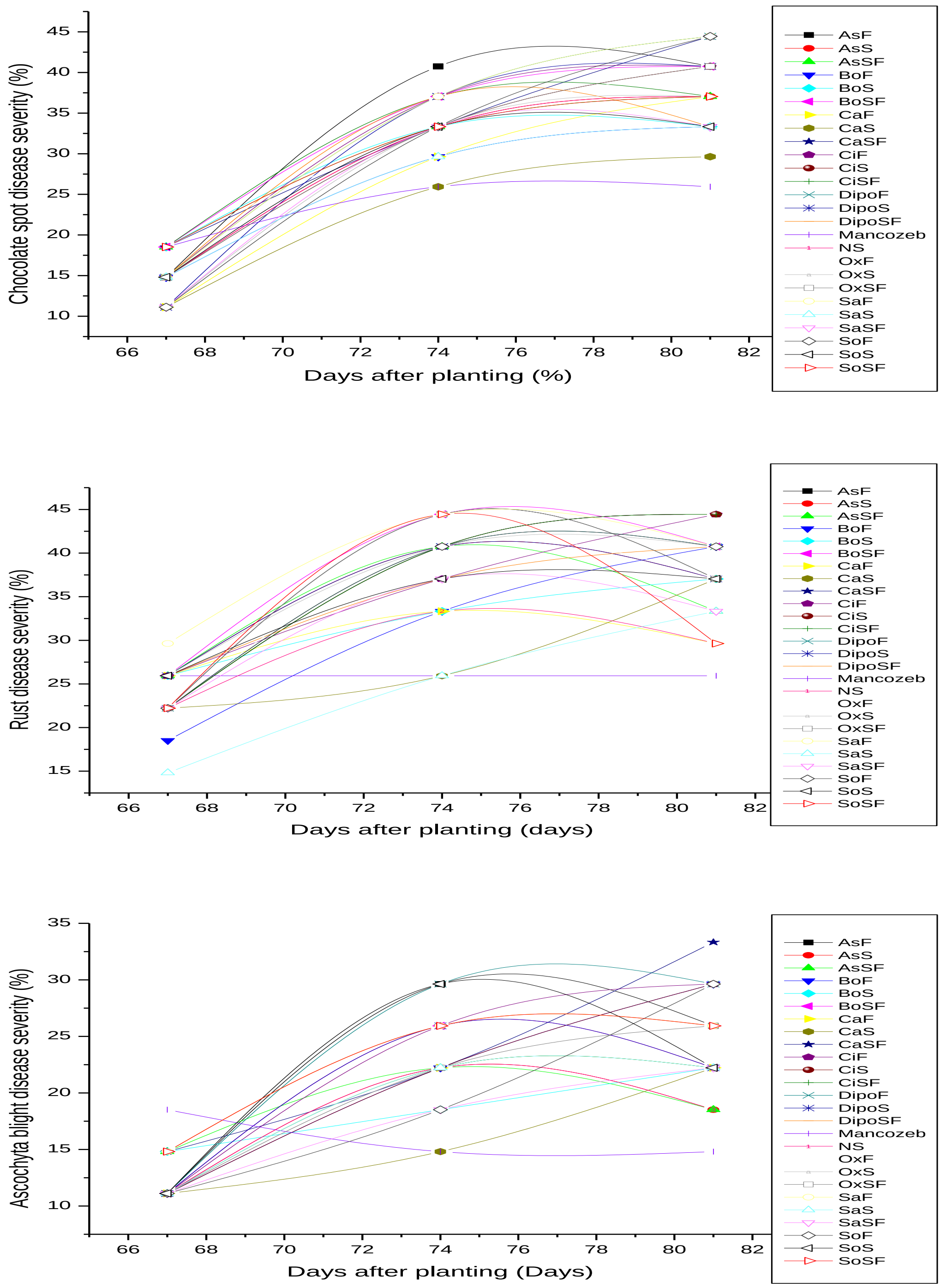


**Figure 1: Influence of signaling chemicals on chocolate spot (A), Rust (B) and Ascochyta blight (C) disease severity**

**Table 2: Effect application of signaling chemicals on Chocolate spot, Rust and Ascochyta blight diseases development**

| Treatment | Chocolate spot (%) | Rust (%) | Ascochyta blight (%) |
|---|---|---|---|
| Salicylic Acid ($C_7H_6O_3$) applied as seed treatment | 25.9 | 24.7 | 18.5 |
| Oxalic Acid ($C_2H_2O_4$) applied as seed treatment | 27.2 | 35.8 | 18.5 |
| Sodium Carbonate ($NaCO_3$) applied as seed treatment | 27.2 | 33.3 | 21 |
| Ascorbic Acid ($C_6H_8O_6$) applied as seed and foliar treatments | 29.6 | 33.3 | 18.5 |
| Salicylic Acid ($C_7H_6O_3$) applied as foliar treatment | 32.1 | 38.3 | 22.2 |
| Di-Potassium hydrogen phosphate ($K_2HPO_4$) applied as seed and foliar treatments | 28.4 | 34.6 | 22.2 |
| Calcium chloride ($Ca\ Cl_2$) applied as foliar treatment | 25.9 | 29.6 | 18.5 |
| Oxalic Acid ($C_2H_2O_4$) applied as seed and foliar treatments | 29.6 | 34.6 | 19.8 |
| Boric Acid ($H_3BO_3$) applied as seed and foliar treatments | 32.1 | 37.0 | 19.8 |
| Di-Potassium hydrogen phosphate ($K_2HPO_4$) applied as seed treatment | 29.6 | 34.6 | 19.8 |
| Calcium chloride ($Ca\ Cl_2$) applied as seed and foliar treatments | 32.1 | 35.8 | 23.46 |
| Citric Acid ($C_6H_8O_7$) applied as seed and foliar treatments | 30.9 | 35.8 | 18.5 |
| Ascorbic Acid ($C_6H_8O_6$) applied as foliar treatment | 32.1 | 34.6 | 22.2 |
| Boric Acid ($H_3BO_3$) applied as foliar treatment | 25.9 | 30.9 | 21 |
| Boric Acid ($H_3BO_3$) applied as seed treatment | 28.4 | 32.1 | 18.5 |
| Sodium Carbonate ($NaCO_3$) applied as seed and foliar treatments | 29.6 | 32.1 | 22.2 |
| Citric Acid ($C_6H_8O_7$) applied as foliar treatment | 30.9 | 35.8 | 22.2 |
| Calcium chloride ($Ca\ Cl_2$) applied as seed treatment | 22.2 | 28.4 | 16 |
| Untreated control | 28.4 | 28.4 | 17.3 |
| Di-Potassium hydrogen phosphate ($K_2HPO_4$) applied as foliar treatment | 32.1 | 34.6 | 23.46 |
| Mancozeb 80% WP applied as foliar treatment | 23.5 | 25.9 | 16 |
| Oxalic Acid ($C_2H_2O_4$) applied as foliar treatment | 30.9 | 33.3 | 19.8 |
| Salicylic Acid ($C_7H_6O_3$) applied as seed and foliar treatments | 25.9 | 30.9 | 17.3 |
| Citric Acid ($C_6H_8O_7$) applied as seed treatment | 29.6 | 35.8 | 21 |
| Ascorbic Acid ($C_6H_8O_6$) as seed treatment | 28.4 | 34.6 | 17.3 |
| Sodium Carbonate ($NaCO_3$) applied as foliar treatment | 29.6 | 34.6 | 19.8 |
| **CV (%)** | **15.15** | **12.75** | **17.97** |
| **$LSD_{0.05}$** | **7.14** | **6.91** | **5.83** |

There were also statistically significant ($P \leq 0.05$) differences between treatments for agronomic performance of faba bean (Table 3). The maximum number of tiller per plant (5.6) was counted from a plot treated with Salicylic acid as seed treatment. The most probable reason is that the majority the seeds treated by Salicylic acid have lost their viability and few seeds germinated have got large free spaces without competition. As a result, it has produced the maximum numbers of tillers per plant. The same result was reported by different researchers that salicylic acid inhibits the seed germination and seedling growth of faba bean (Elwakil *et al.,* 2016; Lv *et al.,* 2020). The rate of seed germination inhibition increases with increasing concentration of salicylic acid (Elwakil *et al.,* 2016). The small number of tillers per plant (1.1) was recorded from plot treated with Sodium Carbonate ($NaCO_3$) as seed treatment.

Large number of pods per plant (57.2) was counted from plot treated with Salicylic acid as seed treatment and the lowest number of pods plant (6.2) was recorded from plots treated with Di-Potassium hydrogen phosphate as foliar spray (Table 3). In case of seeds per plant, the highest number (118.8) was counted from plots treated with Salicylic acid as seed treatment and the smallest number of seeds (15) was recorded from plots treated with Di-Potassium hydrogen phosphate as foliar spray. In case of biomass yield, high biomass yield of 1383.3 kg/ha was obtained from plots treated with Calcium chloride as seed treatment. Similar results were published by different researchers. An application of calcium chloride has improved the overall faba bean plant biomass (El-hendawy *et al.,* 2010). The lowest biomass yield of 150 kg/ha was recorded from plot treated with Salicylic acid as seed treatment. Similarly, the highest grain yield of 4008.1 kg/ha was obtained from plot treated with calcium chloride as seed treatment. Calcium chloride has a positive effect on improving the productivity of faba bean. Similar result was reported by El-hendawy *et al.,* (2010). They recorded the highest faba bean grain yield for plots treated by calcium chloride. Whereas the lowest grain yield of 0.6 kg/ha was obtained from plots which have received Salicylic acid as seed treatment (Table 3). This is mainly because of the fact that salicylic acid has got seed germination inhibitory effect which has led the germination and growth of few seeds and seedlings per plot as already reported by different researchers particularly, the inhibitory effect increases with increasing concentration of the salicylic acid and the soaking time (Elwakil *et al.,* 2016; Lv *et al.,* 2020)). This condition has led to the harvest of the smallest seed yield per plot.

**Table 3: Effect of application of signaling chemicals on agronomic performance of Faba bean**

| Treatment | No. of Tiller/plant | No. of Pod/plant | No. of Seed/plant | Plant height | Biomass (kg/ha) | TKW (g) | Grain yield (kg/ha) |
|---|---|---|---|---|---|---|---|
| Salicylic Acid ($C_7H_6O_3$) applied as seed treatment | 5.6 | 57.2 | 118.8 | 8.3 | 150 | 514 | 0.6 |
| Oxalic Acid ($C_2H_2O_4$) applied as seed treatment | 1.8 | 9.7 | 24.7 | 89.3 | 816.7 | 504.9 | 2178.3 |
| Sodium Carbonate ($NaCO_3$) applied as seed treatment | 1.3 | 7.7 | 17.9 | 91.7 | 1083.3 | 515.2 | 3524 |
| Ascorbic Acid ($C_6H_8O_6$) applied as seed and foliar treatments | 1.7 | 10.4 | 25.4 | 90 | 913.3 | 506.6 | 3411.9 |
| Salicylic Acid ($C_7H_6O_3$) applied as foliar treatment | 2.1 | 13.6 | 31.1 | 97 | 1133.3 | 495.8 | 3404.4 |
| Di-Potassium hydrogen phosphate ($K_2HPO_4$) applied as seed and foliar treatments | 1.4 | 10.4 | 24.1 | 89 | 1000 | 492.5 | 3330.8 |
| Calcium chloride ($Ca\ Cl_2$) applied as foliar treatment | 1.6 | 10.4 | 28.1 | 98.4 | 1033.3 | 494 | 2973.3 |
| Oxalic Acid ($C_2H_2O_4$) applied as seed and foliar treatments | 1.9 | 15.3 | 32.9 | 94.3 | 1046.7 | 501 | 3045.6 |
| Boric Acid ($H_3BO_3$) applied as seed and foliar treatments | 1.3 | 8.7 | 19.6 | 92.6 | 866.7 | 511 | 3221.9 |
| Di-Potassium hydrogen phosphate ($K_2HPO_4$) applied as seed treatment | 2 | 8.6 | 22.8 | 99 | 983.3 | 485.5 | 3169.6 |
| Calcium chloride ($Ca\ Cl_2$) applied as seed and foliar treatments | 1.9 | 10.1 | 27.2 | 87.9 | 1000 | 474.5 | 3073.8 |
| Citric Acid ($C_6H_8O_7$) applied as seed and foliar treatments | 2.3 | 14.1 | 35.7 | 97.2 | 1166.7 | 483.2 | 2791.7 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Ascorbic Acid ($C_6H_8O_6$) applied as foliar treatment | 1.6 | 7.4 | 17.2 | 91 | 883.3 | 466.2 | 2516.7 |
| Boric Acid ($H_3BO_3$) applied as foliar treatment | 2.3 | 12.3 | 29.2 | 97 | 1233.3 | 498.3 | 3156 |
| Boric Acid ($H_3BO_3$) applied as seed treatment | 1.6 | 13.3 | 31.6 | 97.1 | 1366.7 | 485.93 | 3936.9 |
| Sodium Carbonate ($NaCO_3$) applied as seed and foliar treatments | 1.1 | 8.4 | 22.6 | 93.2 | 833.3 | 488 | 2374.4 |
| Citric Acid ($C_6H_8O_7$) applied as foliar treatment | 1.8 | 8.9 | 21.2 | 89.4 | 1026.7 | 467.4 | 2795 |
| Calcium chloride ($Ca\ Cl_2$) applied as seed treatment | 1.9 | 9.8 | 20.7 | 101.2 | 1383.3 | 508.6 | 4008.1 |
| Untreated control | 1.7 | 8.9 | 21.3 | 92.9 | 1033.3 | 480.9 | 2516.3 |
| Di-Potassium hydrogen phosphate ($K_2HPO_4$) applied as foliar treatment | 1.4 | 6.2 | 15 | 91.2 | 966.7 | 488.9 | 2565 |
| Mancozeb 80% WP applied as foliar treatment | 1.4 | 7.8 | 19.3 | 91.6 | 1050 | 486.7 | 3297.7 |
| Oxalic Acid ($C_2H_2O_4$) applied as foliar treatment | 2 | 15.2 | 36.6 | 95.8 | 1083.3 | 488.7 | 3134 |
| Salicylic Acid ($C_7H_6O_3$) applied as seed and foliar treatments | 5.7 | 37.3 | 70.5 | 91.4 | 180 | 468.5 | 248.1 |
| Citric Acid ($C_6H_8O_7$) applied as seed treatment | 2.2 | 16.2 | 36 | 102.8 | 1150 | 513.3 | 3008.3 |
| Ascorbic Acid ($C_6H_8O_6$) as seed treatment | 1.8 | 10.8 | 25.9 | 100.1 | 1116.7 | 495.5 | 3344 |
| Sodium Carbonate ($NaCO_3$) applied as foliar treatment | 2.2 | 9.3 | 21.6 | 94.1 | 966.7 | 502.7 | 2743.3 |
| **CV (%)** | **38.55** | **39.87** | **33.12** | **10.44** | **24.93** | **6.38** | **23.08** |
| **$LSD_{0.05}$** | **1.30** | **8.75** | **16.3** | **NS** | **400.06** | **NS** | **1072.9** |

## Conclusion and Recommendation

From this study it is understandable that most of the signaling chemicals have positively influenced the diseases pressure on Faba bean and also positively affected the yield potential of the crop. Although, out of the evaluated signaling chemicals, most of them have affected different parameters differently but seed treatment with Calcium chloride have given the highest grain yield of 4008.1 kg/ha; and in general the average grain yield of Faba bean from most of the signaling chemicals was high. This indicates that apart from its induction of SAR, they have also enhanced the yielding potential of the crop. Therefore, from this finding it is recommended that the use of Calcium chloride for the management of faba bean diseases through induced SAR on the crop can be important production package of faba bean production and this chemical can also be important component of IDM in the management of Faba bean diseases.

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