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Effect of SAR Chemical Activators (INA, SA, Vit.B_1) as Pesticide Alternatives on Health And Viability of Barley Seeds

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ABSTRACT: Barley seeds were soaked in chemical concentrations of systemic acquired resistance (SAR) inducers; 2,6-dichloroizonicotinic acid (INA), Salicylic acid (SA), Thiamin (vit.B_1) to test their effect on growth parameters such as; germination percentage(G%), fungal percentage, plumule and radical length, wet and dry weight, Speed Germination Index (SGI), Seedling Vigor Index (SVI) and Seedling health evaluation. Results showed that highest seed G% was for INA (80%), with lowest fungal percentage (7.5%) compared to other treatments (SA and vit.B_1) including seeds soaked in water (control). INA treatment showed also highest values in plumule and radical length (7.26 cm and 8.02 cm) respectively. Wet and dry weight measurements for germinated seeds proved that control treatment has high values compared to SAR chemical activators. In case of seedling measurements grown in pots filled with sand for presoaked seeds results indicated that INA treatment exceeds other treatments in G% of normal seedling with highest value (92.50%), similarly at measurements of plumule and radical length and wet and dry weight. To support growth measurements other parameters were tested to assess seed health and vitality, INA treatment proved again highest values in SGI (31.83), SVI (22.85) and seedling health evaluation (81.25) compared with control and another SAR activators SA and vit. B_1.
Keywords: SAR, chemical activators, Seed-borne fungal diseases

Introduction

Barley, *Hordeum vulgare* L. (Poaceae), is an annual monocotyledonous herb. Belonging to tribe Triticeae, barley is evolutionarily closely related to two other small-grain cereal species, wheat and rye, although the genus *Hordeum* is known to have diverged c. 12 million years ago (von Bothmer and Komatsuda, 2011). Today, barley is a significant crop plant globally, and it is mainly exploited as feed or as a raw material for malt production. In many regions such as Northern Africa and mountainous areas of Asia it is a staple food. Only recently the high content of soluble dietary fiber present in barley and its proven health effects have boosted the status of barley as a food ingredient (Holopainen-Mantila, 2015). Seed-borne pathogens have significant influence on seed production and food industry because they: (i) can affect germination, growth and crop productivity (ii) cause seed and seedling diseases (iii) cause biochemical changes, such as the reduction of carbohydrate, protein and total oil content (iv) cause contamination of grains with mycotoxins that represent a health risk to humans and animals (Levic et al., 2012). Damages such as seed death, seedling and plant abnormalities or decreased seed vigor caused by seed-borne pathogens are not always recognized by users (Mukhtar, 2009). Some of the seed-borne mycoflora of barley include *Alternaria alternata*, *Aspergillus candidus*, *A. flavus*, *Curvularia lunata*, *Drechslera halodes*, *Fusarium moniliforme*, *F. pallidoroseum*, *F.solani* and *Ulocladium sp*., (Fakhrunnisa et al., 2006).

According to (Durrant and Dong, 2004; Cho et al., 2010), systemic acquired resistance (SAR) is one such inducible defense mechanism, which is induced systemically throughout the plant in response to the exposure of another part of the plant to a necrogenic pathogen. Chemicals used as inducers of resistance should have no direct toxicity to pathogens, either by the compound itself or by its possible metabolites (Gozzo, 2003); no toxicity to plants and animals; no negative effects on plant growth, development and yield; broad spectrum of defense; low loading amount; long lasting protection; low economical cost for farmers; good profit for producers (Melvin and Muthukumaran, 2008). Chemical elicitors like salicylic

acid, jasmonic acid, DL-β-aminobutyric acid (BABA), thiamine (vit. B_1), oxalic acid, 2, 6-dichloroisonicotinic acid (INA) and acibenzolar-S-methyl benzo-(1,2,3)-thiadiazole-7-carboxylic acid S-methyl ester (ASM) have been successfully employed in controlling diseases of various crop plants. The induction of resistance by chemical elicitors can form an important component in the integrated plant disease management program (Abdel Razik et al., 2008; Ahn et al., 2007 and Martin et al., 2009).

Due to the lack of data concerning of surveying fungi associated with barley grain in Libya, and regarding to a few studies on the induction of systemic acquired resistance in barley seeds, this study comes as an attempt to find out the possibility of the induction of systemic resistance in barley grains presoaking in soluble concentrations of chemical inducers and how this inducible resistance transforming to barley seedlings.

Experimental

Barley grain source: barley local variety grains were purchased from Agricultural equipment stores in Suluq region local marked. 400 barley homogenous grains has been counted and were surface sterilized in 1% sodium hypochlorite solution for 5 min. then rinsed with sterilized water and air dried.

Source of chemicals and preparations: Three chemical inducers; Salicylic acid (SA) Sigma-Aldrich chemie-France**,** Isonicotinic acid (INA), Thiamine hydrochloride (vit.B_1), Sigma-Aldrich chemie-Germany, were purchased from Sigma-Aldrich chemicals company-Cairo branch. Each chemical was dissolved in 50 ml dH_2O or ethyl alcohol, and adjusted to pH 7 with 1N NaOH. Four pre-soaking solutions were prepared as: H_2O (control), INA (10 ppm), vit.B_1 (10 ppm), SA (10 ppm).

Seed treatment and sowing (blotter test): 100 barley grains were soaked in each chemical concentration and H_2O (control) for 6 hrs. then rinsed with sterilized water and air dried. 10 barley seeds were sowing in Petri dishes (9 cm) on three layers of filter paper in three replicates for each treatment including H_2O (control). Distilled water was added to each Petri dish, during the experiment as required. Dishes were incubated at 25°C±2 for seven days under alternating cycles of 12 h light and darkness.

Seed sowing in plastic pots: 14 cm diameter plastic pots were used. Pots were sterilized by sub-immersing in 7% formaldehyde solution for few hours and then left to aerate. The sandy soil was washed repeatedly, sterilized in oven at 160 °C and left to aerate. Pots were filled with sandy soil and sowing at the rate of 10 seeds per pot in three replicates for each treatment including H_2O (control) and were placed in the greenhouse at 12:12 hours light: dark cycle, with 24-26 ˚C: 16-18 ˚C day: night temperature and about 65% relative humidity.

Plumule and radical length: Measurements of emerging plumule and radical for the seeds grown in Petri plates were taken from the second day until the seventh day of planting. While seeds grown in pots their measurements were taken after 14 days by transparent ruler. 1mm considered as indicator of the germination of the seed.

Wet and dry weight: plumules and radicals were separated for each dish or pot separately. Wet weights were done by sensitive digital balance then placed within folds of aluminum foil and dried at 100 °C, dry weight was done by the same balance.

Fungal identification and percentage of incidence: Seed-borne fungi were identified according (Ellis, 1971; Williams-Woodward, 2001). Percentage of existed fungi on treated seeds in Petri plates was calculated after 7 days of incubation by examining the seeds under the Stereo-binocular microscope and recorded as follows:

Fungal (%) = $\frac{n1}{n2} \times 100$ where, n_1 the seeds with fungal growth and n_2 the number of treated seeds.

Germination percentage (G %) (normal seedlings): It was calculated by counting only normal seedlings (ISTA., 1999) rules:

$$\text{MGP} = \frac{n1-n2}{n1} \times 100$$

Where, MGP is the mean percentage, n_1 is number of treated seed plated and n_2 the number of abnormal seedlings plus died seeds

Speed Germination Index (SGI): It was calculated as described in the (AOSA., 1983) by the following equation:

$$\text{SGI} = \frac{\text{No.of germinated seed}}{\text{Days of first count}} + \frac{\text{No.of germinated seed}}{\text{Days of final count}}$$

Seedling Vigor Index (SVI): At the final count, ten normal seedlings from each replicate were randomly taken to measure seedling dry weight (g) (ISTA., 1999) rules: **(SVI)** = MGP × seedling dry weight

Seedling health evaluation: The seedling test were separated into abnormal seedlings, seed rot as described in the technical bulletin on seed-borne diseases (Agarwal

et al., 1989) and applying the formula described by (Ibrahim, 2015):

Fungal infection (%) =

$$\frac{\text{No.of fungal infection on abnormal seedlings or seed rot}}{\text{Total of abnormal seedlings or seed rot}} \times 100$$

Statistical analysis: The observed data was statistically analyzed as the technique of analysis of variance ANOVA of the randomized complete block design as mentioned by (Gomez and Gomez, 1984). The means were compared using the Least Significant Differences (LSD). Statistical analysis was performed using NCSS (2007) computer software package.

Results and discussion

Fungal identification and percentage of incidence: Germination percentage and fungal (%) of barley seeds grown in Petri plates pretreated with SAR chemical inducers Table 1 showed that highest germination percentage resulted in INA inducer (80%) followed by SA (76.66%), then the control treatment (73.33%), and finally vit. B_1 inducer (70%). When treated and untreated barley seeds tested for fungal incidence, highest fungal % was in control (seeds soaked in water) with (41%) followed by vit. B_1 treatment (17.5%), then SA treatment (16.6%) and finally INA with least percentage (7.5%).

Table 1: Germination percentage, fungal incidence and identified fungi on barley seeds grown in Petri plates presoaked with SAR inducers.

Treatments	Germination %	Fungal %	Identified fungi
Control	73.33	41	*Alternaria alternata, Ulocladium , Rhizoctonia*
Vit.B_1	70	17.5	*Alternaria alternata, Ulocladium*
INA	80	7.5	*Rhizoctonia*
SA	76.66	16.6	*Alternaria alternata*

Results of fungal incidence show that the seeds treated with INA gave the best results in terms of percentage of germination 80% and the lowest percentage of fungal incidence 7.5% compared to control, which shows the largest percentage 41%, which indicate its importance as a pivotal role in the emergence of resistance to fungi and reduce their incidence. The current study showed that seeds presoaking with systemic resistance inducers SA, INA and vit.B_1 gave a positive effect in reducing the incidence of seed-borne fungi and encouraging growth measurements such as germination percentage, for treated seeds compared to control. (Morsy et al., 2011) and (Ibrahim and Keshk, 2014) found similar results. The low rate of germination of untreated grains may be due to the effect of fungus on the internal tissues and their negative effect on the embryo, which reduces germination rate, and found that the competition of fungi with grains on the amount of oxygen in growth medium is related to germination rates (Al Jawhari, 2012). The effect of chemical inducers on resistance to plant disease control is summarized as follows: 1) act as secondary signals in stimulating host plant defense mechanisms (Geetha and Shetty, 2002); 2) activates resistance by increasing the activity of peroxidase enzyme (Hassan et al., 2007); 3) activated resistance by inhibition of certain antioxidant enzymes and catalase (Radwan et al., 2008); 4) Stimulate resistance by direct effect on the multiplication and progression of pathogens or indirect effect on metabolism by successive effects on pathogen food supply (Khan et al., 2003). Effect of resistance agents on fungal disease incidence and severity may be explained by increasing the endogenous SA levels in plants, and by induction of pathogenesis related proteins (PRs), thereby initiation of systemic acquired resistance in plant tissues. This agreed with what many research studies have indicated on the role of external application of this SA (Atiq et al., 2013). Our findings agreed with (Ragab et al., 2009) who immersed basil seeds in different concentrations of salicylic acid, oxalic acid and ascorbic acid with concentrations of 8.4,2 millimoles, respectively, against rotten basil disease *Rhizoctonia solani* that have been very effective in reducing the incidence of root rot and increase the proportion of healthy plants.

Plumule and radical lengths: measurements of plumule length in Petri plates for barley seeds pretreated with SAR inducers shown in Table 2 that INA and SA treatments (7.26, 7.12 cm), respectively exceed control (6.92 cm), while vit.B_1 treatment (6.63 cm) decreased than control, however, the differences between control and other treatments were insignificant.

Table 2: Plumule and radical lengths (cm) for barley seeds grown in Petri plates pre-treated with SAR inducers.

Treatments	Plumule and Radical lengths for plated seeds	
	Plumule length (cm)	Radical length (cm)
Control	6.92	7.95a
Vit.B_1	6.63	7.52ab
INA	7.26	8.02a
SA	7.12	6.84b
LSD	0.98 (N.S.)	0.94

Values followed by the same letter(s) in each column don't differ significantly according to LSD test ($P \leq 0.05$).

For radical measurements INA (8.02 cm) proved again superiority in radical length against other treatments but with insignificantly with control and vit.B_1. SA treatment showed least radical length (6.84 cm).

Plumule and radical wet and dry weights: wet and dry weight measurements of plumule and radical grown in Petri dishes Table 3 showed that control plumule exhibited greater wet weight (0.93 g.) close to INA treatment (0.90 g.) and vit.B_1 (0.86 g.) without significant differences with control, while SA treatment (0.83 g.) least than control significantly. Radical wet weight vit.B_1 and INA (0.90, 0.83 g.) exhibited no significant differences with control, while SA (0.63 g.) decreased than control significantly. Greater values for plumule and radical dry weight were in control treatment (0.082 and 0.102 g.) respectively, while other inducer treatments vit.B_1, INA, SA exhibited closer weights either for plumule or radical insignificantly and less than control.

Table 3: Plumule and radical wet and dry weight (g.) for barley seeds grown in Petri dishes pre-treated with chemical inducers

Treatments	Wet weight (g)		Dry weight (g)	
	Plumule	Radical	Plumule	Radical
Control	0.93a	0.90a	0.082a	0.102a
Vit.B_1	0.86ab	0.90a	0.072b	0.085ab
INA	0.90ab	0.83a	0.075ab	0.081b
SA	0.83b	0.63b	0.074ab	0.070b
LSD	0.13	0.08	0.01	0.020

Values followed by the same letter(s) in each column don't differ significantly according to LSD test ($P \leq 0.05$).

Growth studies in Pots: Barley seeds presoaked in SAR inducers and water in addition to their development in Petri dishes, the seeds were grown in plastic Pots containing sand for two weeks, then measurements of growth percentage of seedlings, shoot and root lengths, seedling wet and dry weight, speed germination index, seedling vigor index and seedling health evaluation were tested. Results in Table 4 showed that normal seedling G% of the seeds presoaked in INA was with higher value 92.5% compared to control (water soaked) 58.33%, also vit.B_1 and SA treatment exceed control by 82.5% and 83.33%, respectively.

Table 4: Germination percentage of normal seedlings from grown barley seeds in Pots presoaked in SAR inducers and water.

Treatments	G% of barley normal seeds
Control	58.33
Vit.B_1	82.50
INA	92.50
SA	83.33

Seedling measurements: for seedling shoots and roots it is clear from Table 5 that INA treated seeds gave longest shoot and root 17.29 cm and 14.89 cm, respectively, compared to control, while other treatments, their measurements converge with control insignificantly in shoot length, but in root length control treatment less than all SAR inducers with no significant differences except for vit.B_1 treatment. In the case of wet and dry weights for seedlings grown in Pots Table 5 showed that INA has the heaviest wet and dry weights 3.56 g and 0.247 g compared to control and other treatments but insignificantly. Vit.B1 treatment exhibited least wet and dry weights 2.80 g. and 0.228 g. than control.

Table 5: Seedlings measurements; shoot and root lengths (cm), wet and dry weights (g.) for barley seeds grown in Pots pre-treated with chemical inducers

Treatments	Seedling length (cm)		Seedling weight (g.)	
	Shoot	Root	Wet weight	Dry weight
Control	16.63	13.34a	3.36	0.230
Vit.B_1	16.97	10.81b	2.80	0.228
INA	17.29	14.89a	3.56	0.247
SA	16.39	13.86a	3.13	0.235
LSD	(N. S.)	2.00	(N. S.)	(N. S.)

Values followed by the same letter(s) in each column don't differ significantly according to LSD test ($P \leq 0.05$).

For more evaluation of SAR inducers and its role in plant protection, other points have been adopted such as; speed germination index (SGI), seedling vigor index (SVI) and seedling health evaluation. Results in Table 6 indicated that a barley seeds presoaked in INA solution has highest values in SGI (31.83) and SVI (22.85) compared to control and other treatments. At measuring of seedling health evaluation INA also exhibited highest value (81.25), while other treatments (SA, vit.B_1) values were least than control (69.23). It was clearly from this evaluation that the response of INA in accelerated germination of barley seeds and strengthens seedling vigor, in addition to maintain the seedling health from fungal infection.

Table 6: Speed germination index (SGI), seedling vigor index (SVI) and seedling health evaluation for barley seeds pre-treated with SAR inducers in Pot experiment.

Treatments	Speed germination index	Seedling vigor index	Seedling Health evaluation
Control	28.83	13.42	69.23
Vit.B1	26.66	18.81	54.54
INA	31.83	22.85	81.25
SA	27	19.58	58.33

Results of current study were concerning with plumule and radical lengths, their wet and dry weights for seeds in Petri dishes and seedlings in Pots, these results were agreed with many previous studies such as; (Nighatsarwar et al., 2005) in which chickpea seeds soaked for 24 hrs with different concentrations of SA led to increase of wet and dry weight compared to control, and that of (Mohammadi, 2009) in which seeds treated with potassium nitrate gave higher values compared to control in germination percentage, germination rate and dry weight of the seedling, and (Ahmadvand et al., 2012) who found that germination percentage, plumule and radical, plant height, leaf area, dry weight of the plants produced from treated seeds that their measurements were high in comparison to the untreated seed plants. Our findings proved that SA treatment had least G% in Petri dishes compared to other inducers that agreed with findings of (Madah, 2005) in which low concentrations from SA increases G%, but this insignificant compared to control. Our results showed a decrease of SA on plumule and radical wet and dry weights, this is contrary to (Baghizadeh and Hajmohammadrezaei, 2011) and (Al-Hakimi and Saeed, 2007) where they found that SA application increases wet and dry weights of plumule and radical. Results showed also decrease effect of vit.B_1 treatment on SGI, SVI and seedling health evaluation, however, (Neumann et al., 1996) demonstrated that immersing seeds in thiamine increase significantly growth rate in beans, it also reduces affected and abnormal seedlings. (El-Mahady et al., 2015) proved the importance of presoaking of seeds in SA in seedling vigor. Our finding indicated that application of SAR inducers increase growth parameters, SGI, SVI and seedling health evaluation, though at varying rates, this was similar to many studies such as; (Afzal et al., 2005; Hayat et al., 2005; Shakirova, 2007 and Saharan and Nehra, 2011).

Conclusion

In this work, three chemicals were environment friendly products serve as pesticides alternatives especially INA where it was more effective in SAR induction, regarding to all growth parameters; Germination %, Fungal %, plumule and radical measurements, seedling measurements. Additional measurements such as; speed germination index, seedling vigor index and seedling health evaluation approved that INA is a major component in systemic acquired resistance and integrated disease management programs.

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