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EFFICACY OF ECO-FRIENDLY COMPOUNDS IN ENHANCING BIOCHEMICALLY MANGO RESISTANCE TO POWDERY MILDEW DISEASE

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Abstract

Mango (*Mangifera indica* L.) is considered one of the most important fruit for millions of people worldwide, particularly in Egypt where it is deemed to be the choicest of all indigenous fruits. Mango powdery mildew caused by *Oidium mangiferae* Berthet is a severe disease that infects all parts of mango trees except the fruits. The present investigation aimed to evaluate the use of some eco-friendly compounds as alternatives to control the disease.

The obtained results indicate that acetic acid at a concentration of 16.5ml/liter proved to be the most effective eco-friendly compound in decreasing mango powdery mildew disease incidence resulting from the highest efficiency percent in both investigated seasons compared with the tested fungicide propiconazole.

Mango plants indicate the highest proline, phenols and protein contents when treated with sulfur. Sulfur was also an inequality with all the other investigated eco-friendly compounds in enhancing oxidative enzyme activities.

Key words : Mango, *Oidium mangiferae*, eco-friendly, oil and plant enzymes, antioxidants.

Introduction

Powdery mildew caused by a biotrophic fungus *Oidium mangiferae* Berthet is the most serious disease throughout the world and in Egypt (Nofal and Haggag, 2006 and Muhammad *et al.*, 2014). Mango is the only known host of this pathogen (Reuveni *et al.*, 2018).

Mango powdery mildew can be controlled by several types of control, one of them depended on using environmentally friendly natural compounds and some bio-agents, however, the other ways depended on fungicides, were used. The use of some natural substances including salts and vegetable oils in disease control are recommended by several investigators as types of safe ones (Nofal and Haggag 2006 and Akhtar *et al.* 1998). Nofal and Haggag (2006) found that environmentally friendly compounds like mineral salts (phosphate solutions, anti-transparent, and kaolin) and antioxidant (ascorbic acid) were effective in reducing mango powdery mildew singly or in mixtures as alternatives to chemical fungicides.

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Mango foliar sprays with MKP, KHPO + KOH and commercial systemic fungicides inhibited the development of powdery mildew on flower, fruit, and leaves (Reuveni and Reuveni, 1995). Also, (Napier and Oosthuyse, 1999) reported that MKP sprayed alone or in alternation with fungicides, successfully controlled powdery mildew in many types of fruit and mangoes. In most cases, the conventional fungicide programmer may be cut by 50%. The effects of MKP as a biocompatible fungicide appears to be synergistic with that of the fungicide. As well as Hsieh *et al.*, (2005) found that spore germination of *Oidium neolycopersici, Erysiphe pisi,* and *Sphaerotheca pannosa* was significantly suppressed by three bicarbonates, especially sodium and potassium bicarbonate.

Crisp *et al.,* (2006) showed that potassium bicarbonate at 3gm/l reduced powdery mildew disease severity with a maximum value of 96% compared with untreated control.

Oosthuyse (2007) showed that potassium dihydrogen phosphate was effective in suppressing powdery mildew of mango.

Sawant and Sawant (2008) showed that potassium bicarbonate alone was effective at 10 g/L when given as preventive sprays at 10 days interval starting from 40 days after pruning and it also reduced sporulation of powdery mildew when sprayed on infected leaves at 10g/L. They added that potassium bicarbonate was found compatible with myclobutanil, flusilozole, and pencnazole and can be used in combination with these fungicides.

Plant treated with potassium bicarbonate and potassium phosphate showed less severity of powdery mildew (*Oidium* sp.) than the controls. The results also showed that potassium phosphate and potassium bicarbonate at 5 and 4.7 g/L respectively, reduced significantly the incidence and severity of the disease (Yanez Juarez *et al.,* 2012).

Both the biological processes and the chemical content of the plant are affected when exposed to pathogens or exposure to certain treatments. The total content of phenol, protein, proline, and activity of oxidative enzymes may be change in plant metabolism.

Salysillic acid (SA) treated melon plants significantly lowered powdery mildew than that of control. Treatment with SA could increase the contents of photosynthetic pigments and activities of antioxidant enzymes and increased resistance to powdery mildew (Wang, 2008).

Ashry and Mohamed (2012) mentioned that the activity of peroxidase, polyphenyl oxidase, catalase enzymes as well as proline content was significantly increased in powdery mildew infected leaves. Tamas and Huttova, (1996) found that in potassium phosphate sprayed leaves, the protein accumulation pattern was very similar to that in powdery mildew-inoculated leaves. Also, they found that salicylic acid treatment induced the accumulation of 2 of the 6 pathogen-induced proteins and salicylic acid-specific protein. Inoculation with powdery mildew increased peroxidase, esterase, and acid phosphatase activities. Hegazi and El-Kot (2010) found that the activities of peroxidase (POX) and polyphenoloxidase (PPO) enzymes increased as a result of plant's essential oils spray.

Mahattanatawee *et al.,* (2006) found that antioxidant activity showed high correlations with levels of soluble phenols (TSP). Northover and Schneider (1996) showed that the petroleum oils and glyceridic plant oils were effective in suppressing powdery mildew on potted vines cv. chardonnay under greenhouse conditions.

Beresford *et al.,* (1996) found that the addition of mineral oil had little effect in controlling powdery mildew in apple field trials. Baking soda (sodium bicarbonate) gave slight control of powdery mildew.

Sulfur is very effective as preventing natural product that controlling powdery mildew. Reza and Mortuza (1997) concluded that the prevalence and severity of powdery mildew were found to be reduced effectively by 2 foliar sprays with sulfur at 2000 ppm followed by propiconazole (Tilt) at 500 ppm. The highest fruit retention was also recorded from sulfur treated plants.

Torre *et al.,* (2004) found that good results against powdery mildew on marrows were obtained when using sulfur and potassium permanganate in the first and the second years of the trial, using sulfur alone or in mixture with sodium bicarbonate, and the products tested did not show any phytotoxic symptoms. Also, Bourbos and Barbopoulou (2007) reported that the effectiveness of the sulfur was 97.1% and 98 to 98.1% for sodium bicarbonate on olive powdery mildew *Leveillula taurica*.

Fungicides can be used to treat powdery mildew on mangoes. For optimal effect, the treatment should start before flowering or at a very early flowering stage. Continuous applications at regular intervals of 7-14 days are recommended.

Several fungicides significantly controlled mango powdery mildew and increased fruits yield *i.e.* flusilazole or pyrazophos (Lonsdale and Kotze 1991), Topas (Haq *et al.,* 1994), thiophanat-methyl and sulfur (Akhtar *et al.,* 1998), hexaconazol (Chavan *et al.,* 2009), amistar 25 SC (Fugro *et al.,* 2012), punch (Hemant *et al.,* 2012) and penconazole, myclobutanil, tetraconazole (Reuveni *et al.,* 2018).

Thus, the present investigation aimed to study and evaluate the effectiveness of some alternatives including safe mineral oils, mono-carbonate, bicarbonate, phosphate salts, various antioxidants and sulfur to control mango powdery mildew in order to minimize the hazardous effects of the fungicides.

Materials and Methods

Disease parameters

The diseased leaves and efficiency were calculated using the following scale described by Lonsdale and Kotze (1993) where: 0= panicle free of disease, 1=1-25%infection, 2=26-50% infection, 3=51-75% infection and 4= more than 75% infection.

Application of eco-friendly compounds as resistance inducers

Some natural products have been applied in field experiments to control powdery mildew disease of mango under natural infection. The trial was applied in two growing seasons 2016 and 2018 on the mango trees of 10 years old in fields of Sokary and Zebda cultivars that were cultivated in distances 6x6m between trees in Abu Hammad, Sharqia governorate at Mid of March to Mid of April with 15 days interval at both years tested. Four trees as replicates / each particular treatment were sprayed immediately after the appearance of the first symptoms of powdery mildew on the panicles. Check trees were sprayed only with water. Treatments included mineral oil (paraffin oil), salts (monopotassium phosphate and potassium bicarbonate), salicylic, ascorbic, acetic acids, dish soap and micronized sulfur, were used as foliar sprays application compared to fungicide. The tested concentrations were prepared /1 liter of water as follows: paraffin oil at rate of 2 ml, dish washing soap at 1 ml, mono-potassium phosphate 1.75 & 2.5g/liter), potassium bicarbonate at 4. 7 & 10, salicylic acid 0.25 gm, ascorbic acid 0.5 gm & 1 gm and acetic acid 16.5 ml, liquid dish soap 1ml and micronized sulfur 2 g and Nasr-Zool as propiconazole fungicide at 15 ml.

Foliar spraying has been carried out at three times intervals: 1st was at mid of March the second was at1st of April and the third was at mid of April. Disease severity percentage and efficiency percent were assessed randomly one week after each spraying and averaged. The disease severity percent was estimated as mentioned before.

Biochemical changes of Mango leaves

Determination of total phenolic compounds

After two weeks from the last treatment, a sample of treated mango leaves was collected and 2g leaves of each were used to determine the total contents. Phenolic compounds were determined using methods of analysis described by Snell and Snell (1954).

Samples were homogenized in 15 ml ethanol 95% and boiled for 15 minutes. The homogenate was filtrated using filter paper. A quantity of 0.5 ml Folin-Denis reagent was added to one ml of the alcoholic extract and after 5 minutes, 7 ml saturated sodium carbonate solution was added, shaken and left for ½ hr. Optical density was measured at 750 nm and total phenols were calculated from a standard curve of gallic acid. Obtained data were expressed as the mg gallic acid equivalents per gram of fresh weight basis according to Slinkard and Singleton (1977).

Determination of total proline

After two weeks from the last treatment, a sample of treated mango leaves was collected for proline determination according to the methods described by Batas *et al.,* (1973). The chromophore containing toluene was absorbed from the aqueous phase warmed to room temperature and the absorbance read was at 520 nm on the spectrophotometer system in Quality Control Laboratory in Al Shams Agro Group. Using toluene for a blank the proline concentration was detected according to a standard curve.

Determination of protein content

The digestion process took place in Lab. of Agri., Bot. Dep. Fac. Agri., Zagazig Univ. After two weeks from the last treatment, a sample of treated mango leaves was collected and dried in an oven at 70ºC for 48 hours then 4ml of sulfuric acid and 0.5 ml perchloric acid was added and put on a hot plate. The mixture was converted into black then gradually turns to transparent color which confirms the full digestion. Nitrogen was estimated in every sample in the Central Lab. Fac. Agri., Zagazig Univ. according to the methods followed by Wolf (1982) and Chapman and Pratt (1961).

Determination of antioxidant enzyme activities

The activity of antioxidant enzymes of the plant was evaluated in the Laboratory of Analysis and Measurements lab. of the Central Lab. of Plant Path. Inst. at the Agric. Res. Center.

Phosphate buffer preparation

Eight and nine tenth gram disodium hydrogen was dissolved in 250 ml distilled water in a volumetric flask (solution A) and 3.9 g sodium hydrogen phosphate was dissolved in 250 ml distilled water (Solution B). Solution A was then added to solution B.

Peroxidase activity

One gram of the extracted enzyme sample was crushed well in 2 ml sodium phosphate buffer 0.1 µ at pH 7.1. The homogenate was filtered through filter paper. The suspension was centrifuged at 6000 rpm at 4ºC for 20 min and stored at -18ºC until use.

One-tenth extracted enzyme sample was added to 0.5 ml sodium phosphate buffer 0.1 µ at pH 7.1, 0.1ml H_2O_2 1% and 0.3 ml pyrogallol 0.05 µ. The mixture was completed to 3 ml using distilled water and color density was read in absorbance spectrophotometer Miltonroyspectronic 601 at 425 nm every 30 seconds for 10 reads (Kochba *et al.* 1977). Peroxidase activity was calculated as mg/gm fresh weight.

Polyphenoloxidase activity

Enzyme samples were extracted as mentioned before in peroxidase activity extraction. One-tenth extracted sample was added to 0.5 ml sodium phosphate buffer 0.1

ml at pH 7and 0.5 ml catechol 0.001 N. The mixture was completed to 3 ml using distilled water and color density was read in spectrophotometer Milton Roy Spectronic 601 at 495 nm every 30 seconds for 10 reads (Lisker *et al*., 1983).

Statistical analysis

The previously obtained results were statistically analyzed according to the methods reported by Gomez, (1984) and Duncan, (1955).

Results and Discussions

Using eco-friendly compounds

Ten years old trees of Sokary and Zebda mango cultivars were investigated. Data presented in Table (1) and illustrated by Figs. (1&2) indicate that acetic acid reveal the (80.49 and 81.23), in sulfur (74.79 and 75.33) and SA (72.33 and 71.00) as eco-friendly tested compounds reducing disease incidence and severity likewise to Nasr-Zool propiconazole fungicides (at 0.15 ml/l) significantly decreased mango powdery mildew disease incidence in both investigated seasons (81.13 and 82.32% respectively) compared to non-treated control.

It could be concluded that the application of such eco-friendly tested compounds and fungicides significantly reduced the disease incidence percent and in consequence improve efficiency.

Powdery mildew affects all parts of mango tree causing severe problems in the fruit yields. Although utilization of the fungicides in the most effective way to reduce the disease incidence frequent application leads to severe damage for the tree and its production and resulted in a resistant population of the pathogen. Thus, it must exclude the negative effect of chemical applications by applying different types of such chemicals, using the recommended dose, applying the protective fungicides instead of the eradicator ones and also improving the use of chemicals with a different mode of actions through the season Brent and Holloman, (2007).

Ascorbic acid as an antioxidant employs the highest percent of disease incidence when used at 1g/L being 49.67% reflecting also the least efficiency for disease control being 47.31 % in season 2016. The least disease incidence 18.33% was obtained when acetic acid at a rate of 16.5 ml/L was applied, thus reflecting the highest efficiency of 80.49% of the disease incidence. Results obtained were significantly differed comparing with the control. The obtained results are in agreement with several investigators who stated that the inducer resistance has an important effect in controlling mango powdery mildew disease (Pasini *et al.*, 1997) who reported that wine vinegar at 5 and 10% gave good control of powdery mildew caused by *Sphaerotheca pannosa* var. *rosa* but

Table 1: Effect of some safe products on the severity of mango powdery mildew of 2016 and 2018 growing seasons.

Eco-friendly compound	Concentration gm or ml/L water	Growing season 2016		Growing season2018	
		Disease incidence (%)	Efficiency(%)	Disease incidence (%)	Efficiency(%)
1. Antioxidant					
Salicylic Acid	0.25	25.67 cd	72.33 abc	28.00 e	71.00 ab
Ascorbic acid	0.5	28.33 cd	69.85 d	56.67 bc	40.97 efg
	1	49.67 b	47.13 a	61.00 bc	36.35 fg
Acetic acid	16.5	18.33 d	80.49 a	14.00 e	81.23 a
2. Mineral salts					
Mono potassiumphosphate	1.75	42.67 bc	54.74 cd	30.33 e	59.42 bcde
	2.5	51.33 b	45.65 d	35.67 e	62.92 abcd
Potassium bicarbonate	4	49.33 b	48.53 d	63.33 bc	33.97 fg
	7	51.00 b	45.95 d	54.00 cd	43.69 defg
	10	36.67 bcd	60.79 bcd	49.00 cd	49.07 cdef
3. Oils					
Paraffin oil	2 ml	78.33 a	19.17 e	71.67 b	24.63 g
4. Liquid Soap	1ml	37.00 bcd	70.27 abc	30.67 e	68.03 abc
5. Sulfur	2 gm	23.67 cd	74.79 ab	23.67 e	75.33 ab
6. Fungicides propiconazole	0.15 ml	4.65 e	81.13 a	4.60 g	82.32 a
Control		94		95	

LSD at level 0.05.

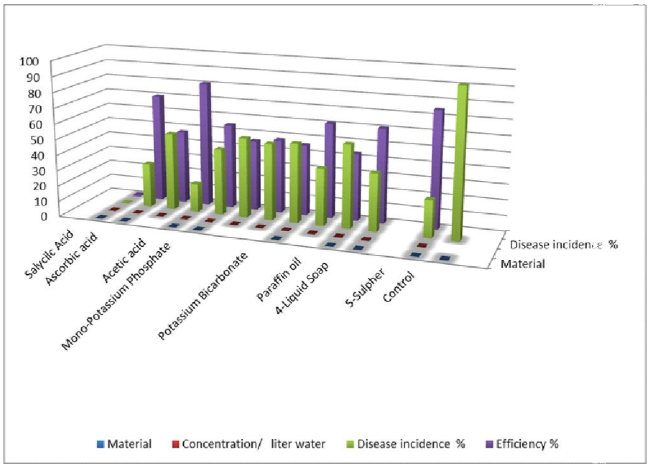

Fig. 1: Effect of some safe products on the severity of mango powdery mildew of 2016 growing season.

acetic acid, was phytotoxic.

Similar trend was obtained in the second investigated season 2018 but with lower different values where acetic acid exhibits the least disease incidence 18.33 % with efficiency being 80.49 %. Also, Ascorbic acid displays the highest value 61% disease incidence reflecting 36.35% efficiency when applied at a rate of 2gm/Liter.

Salicylic acid as a safe product indicates moderate disease inducer at the evaluated concentration 0.25gm in 2016 growing season being 25.67% reflecting mild efficiency 72.33%. In the second season 2018 salicylic acid exhibits higher disease incidence being 28% rather than season 2016.

In general, the obtained data of all the examined chemical inducers *i.e.* salicylic acid, ascorbic acid and / or acetic acid reveal significant effects at their tested concentrations in reducing mango powdery mildew disease incidence if compared with the untreated control.

Data of the same table indicate that all treatments reduced the severity of mango powdery mildew under field conditions as a result of spraying mango plants with solutions of either monopotassium phosphate and or potassium bicarbonate. Both salts display similar disease incidence 51.33% and 51.00% at their concentrations 2.5gm/liter and 7gm/liter for both, respectively, in season 2016. The least value of disease incidence 36.67% was obtained when potassium bicarbonate was investigated reflecting the highest efficiency being 60.79%. Obtained data are in accordance with those reported by Reuveni and Reuveni, (1995), Collina, (1996) and Pasini *et al.*, (1997), Nofal and Haggag, (2006) who reported that natural products have favorable toxicological and environmental characteristics and not phytotoxic and could be used to support the use of sulfur in controlling powdery mildew disease.

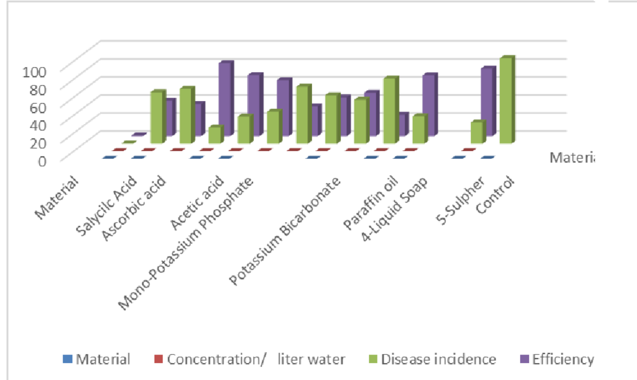

Fig. 1: Effect of some safe products on the severity of mango powdery mildew of 2018 growing season.

Wang (2008) found that systemic acquired resistance (SAR) might be a result of inducing plant resistance which decreases biotic stress on the plant and improve growth promoter's substance. Reuveni and Reuveni (1998) reported that a single application of phosphates was effective in suppressing the lesions of powdery mildew on the diseased mango grown under field conditions.

Data in table 1 also indicate that monopotassium phosphate as an abiotic agent used at the concentration of 1.75g reveals the least value of disease incidence being 30.33% reflecting moderate efficiency 59.42%. However, potassium bicarbonate when sprayed at a concentration of 4 gm reveals the highest percent of disease incidence 63.33% reflecting the least efficiency 33.97% in season 2018.

Application of potassium bicarbonate at 10 gm/Liter appoint the least percent of disease incidence reflecting the highest efficiency being 60.79%. Sodium bicarbonate or phosphate bicarbonate were registered as pesticide ingredients and horticultural fungicide. The physiological mechanism of these inhibiting effects might be due to changes in pH medium which became more alkaline as bicarbonate concentration increased permeability and alter physiological pathways.

Thus, foliar spray of some plant resistance inducers including potassium phosphate and potassium bicarbonate as safe products resulted in a significant reduction percent of powdery mildew disease of mango grown under field conditions. Potassium salts treatments reflected several products of defense signals. The highest records of reduction in the disease incidence were accompanied by an increase in total phenolic, protein contents and increased activity of polyphenol oxidase and peroxidase. Application of potassium salts as natural and chemical inducer by foliar application can be used to control the disease at the early stages of the plant growth that lead to early controlling of the disease and best plant growth.

Paraffin liquid oil is a mineral oil, colorless and odorless that is used for varied purposes. Obtained data of table 1 of the present investigation indicates that paraffin oil has a weak effect in controlling powdery mildew disease incidence of mango where it reveals 78.33% of disease incidence in the first growth season 2016 reflecting the efficiency of 19.17% at a concentration of 2ml/Liter water. Mineral oil alone or in combination with a fungicide suppressed the fungus probably by deactivation of a visible infection and thereby reduced disease level. Mineral oils are distinguished by their high degree of curative and antitransporlative action. In the present work, the weaker effect was observed when mineral oil was applied alone suggests that the oil had a fungistatic rather than a fungicidal effect. Such results are in accorder with with those obtain by Northover and Schnider, (1996). Similarly, mineral oil applied at 7 days intervals demonstrated post-lesion curative and antisporulative action and also it reduced the incidence of disease only marginally.

In growing season 2018 paraffin oil denotes high percentage of disease incidence 71.67 compared with the first growth season 2016. Thus, it must exclude the negative effect of some materials without frequent application.

Liquid dish soap was also examined as shown in table 1, comparing with the other safe products. Liquid soap exhibit low percent of mango powdery mildew disease incidence at the two seasons being 37 and 30.67% when applied at a concentration of 1ml/Liter water reflecting 70.27 and 68.03% efficiency. The explanation of such results might be explained on the basis that such oil might be well distributed only on the surface as mentioned by (Sawant and Sawant, 2008).

As for sulfur when applied as a suspension of 2g it displays very low disease incidence being 23.67% (Table 1) reflecting significant-high efficiency being 74.79%. The same trend was obtained in growing season 2018 where relatively the same results concerning disease incidence percent and efficiency being 23.67% and 75.33, respectively, were calculated. Sulfur usage might provide a beneficial eco-friendly advantage for growers and the environment as it acts as an alternative resistant inducer to fungicides that have the potential to reduce the disease.

The present investigation shows that foliar sprays of sulfur or mineral oils on mildewed tissues suppressed the fungus as demonstrated in a significant reduction in disease incidence in treated trees compared with the untreated ones. This might be due to hyphal deformation and shrinkage of conidia and conidiophers. Sulfur also might act as a preventive by competing for oxygen and interrupting the respiratory chain and as an eradicator, whose phytotoxic action destroy mycelia and kills mycelium filaments, condiophores and established conidia.

Biochemical changes of mango leaves as affected by eco-friendly treatment with some resistant inducers

Phenol contents

Under field conditions, nine different alternatives eco-friendly including three antioxidants, two mineral salts, sulfur, a liquid soap and Paraffin oil were investigated to reduce mango powdery mildew disease and study their effects on the biochemical changes of phenol contents of the treated mango leaves of season 2018.

Data presented in table 2 show that all the tested inducers improve phenol contents in comparison with the control treatment. Application of mono-potassium phosphate at a rate of 2.5g and sulfur at 2 g/L were superior in increasing phenol contents being (3.43mg/g fresh weight of mango) for both inducers, respectively. Potassium bicarbonate at 4g/L reveals the least value of phenol content being 0.58 mg/g fresh weight. However, the increase in the application rate of potassium bicarbonate resulted in a gradual increase in the phenol contents. The remained inducers exhibit values ranging from 0.83 - 2.93mg/g of fresh weight. As mentioned potassium bicarbonate is known for many years with its antifungal properties that act as a contact fungicide (Marku *et al.*, 2014).

Data of the present experiment indicate that spraying mango trees with potassium bicarbonate at 10g/L resulted in an induction of phenol contents comparing with the control and other alternatives i.e. salicylic acid, acetic acid, mono-potassium phosphate at 1.75g/L and paraffin oil.

Ascorbic acid at 1g/L and liquid soap at 1ml/L showed also a remarkable increase in phenol contents being 2.73 and 2.93mg/g fresh weight, respectively.

In general, most of the inducer resistance has an important role in controlling plant disease (Ibrahim *et al.*, 2013 and Muhammad *et al.*, 2014). Mono-potassium phosphate and sulfur as previously mentioned prove to be superior in increasing phenolic contents that might lead to a reduction in the disease severity of mango powdery mildew by enhancing activities of the related phenolic contents and might also affect and activate pathogenesis-related proteins.

Proline contents

Proline is an amino acid used in the biosynthesis of

Table 2: Biochemical changes of mango leaves as affected by some eco-friendly resistant inducers of season 2018.

Material	Concentration g-ml/liters water	Antioxidant enzymesactivity		Proline contents µg/g fresh weight	Phenols contents µg/g fresh weight	Protein ratio%
		Po Δ425	PPO Δ495			
1. Antioxidant						
Salycilc acid	0.25	1.79	0.016	14.76	1.25	2.2
Ascorbic acid	0.5	1.65	0.003	22.10	1.28	1.9
	1g	1.51	0.015	23.90	2.73	1.8
Acetic acid	16.5	1.75	0.004	9.03	0.88	1.9
2. Mineral salts						
Monopotassium phosphate	1.75	1.61	0.003	19.66	2.12	2.1
	2.5	1.72	0.004	23.90	3.43	2.0
Potassium bicarbonate	4	1.81	0.029	21.00	0.58	2.2
	7	1.53	0.009	20.75	0.83	1.9
	10	1.50	0.002	15.00	2.33	2.1
3. Oils						
Paraffin oil	2	1.56	0.031	19.93	2.25	1.8
4. Liquid soap	1	1.67	0.004	23.54	2.93	1.3
5. Sulphur	2	1.62	0.016	3.42	3.43	1.8
Control		1.25	0.001	1.49	0.46	0.4

plant proteins plays an important role in the protection of plants against biotic and abiotic agents.

The present results indicate the highest value of Proline contents being 23.9µg/g fresh weight when mono-potassium phosphate at concentration 2.5g/L water and ascorbic acid 1g/L, were applied, for both. Liquid soap reveals also high contents being 23.54µg/g fresh weight. However, sulfur as a resistant inducer displays the least value 3.43µg/g of fresh weight.

The remained non-fungicidal compounds indicate moderate values ranging from 22.1 to 9.03 µg/g of fresh weight.

In this respect Tie Lin *et al.,* (2016) reported that proline content and antioxidant enzymes are thought to be associated with maintaining the structure of cellular components or with protecting cellular functions against biotic and abiotic agents. Similar results were also obtained by Barnett and Naylor (1966) and Ashry and Mohamed (2011).

Protein contents

Accumulation of proteins of mango leaves suffering from infection with powdery mildew causal organism was determined and calculated. The highest protein ratio percent was recorded when mango plants were treated with salicylic acid 0.25g-ml/L water and/or Potassium bicarbonate at 4g/L being 2.2 for both followed by mono-potassium phosphate at a concentration rate 1.75g/L and potassium bicarbonate at a concentration of 10g/L. Mono-potassium phosphate at a rate of 2.5g/L exhibit also the high value of protein percent being 2%. However, liquid soap exhibit the least value 1.3%. On the other hand, untreated check plants show the least ratio of protein being 0.4. The remained applied inducers i.e. potassium bicarbonate at 7g/L, ascorbic acid at both applied rates, acetic acid, sulfur, and paraffin oil resulted moderate values being 1.9, 1.9, 1.8, 1.9, 1.8 and 1.8%, respectively.

Pathogenic fungal genera cause various plant diseases especially those of the obligate ones that usually weaken or destroy plant tissues and reduce the biochemical metabolism of the host (Bajoria *et al.,* 2008).

Powdery mildew disease cannot be prevented only by the development of resistant cultivars. Although promising results obtained by chemical treatments, phytotoxicity is a major problem leading to environmental pollution and human hazards (Mandal *et al.,* 2009). Thus, the application of non-fungicidal alternatives should be applied to decrease hazards and have been extensively used for the improvement of plant growth and disease control.

Salicylic acid is a phenolic compound that affects a variety of biochemical events associated with induction of disease incidence and it has been reported to play an important role in developing systemic induced resistance after an initial pathogen attack (Saikia *et al.,* 2003).

The present study might provide evidence that might facilitate applying non-toxic chemical *i.e.* salicylic acid, potassium bicarbonate, and other chemicals to share in controlling powdery mildew disease with least toxicity to the plant, environment, and human health through changing plant biochemical metabolism to improve the systemic acquired resistance of the plant.

Oxidative enzyme activities

An Experiment has been conducted under field conditions where mango tree was sprayed with different eco-friendly products *i.e.* antioxidants, mineral salts, paraffin oil, sulfur, and liquid soap.

The results of Table (2) indicate the effect of all the investigated inducers on oxidative enzyme activities. Results indicate that the mineral salt potassium bicarbonate at a concentration of 4gm/L water display a remarkable increase in both Peroxidase (PO) and polyphenol oxidase (PPO) activities being 1.81 and 0.029 as compared with the untreated control. Salicylic acid indicate also considerable level of peroxidase activity being 1.79. On the other hand, acetic acid as an inducer reveal high level being 1.75. However, paraffin oil seemed to be highly inducer of polyphenol oxidase enzyme activity at the tested concentration 2ml/L being 0.031 and also has considerable effect in inducing Peroxidase enzyme activity being 1.56.

Resistance induced by the previously mentioned effective inducers has a wide spectrum against the pathogenic fungus *O. mangiferae* but rarely provides complete inhibition for the pathogen. The application of potassium bicarbonate and salicylic acid as chemical inducers might be considered promising tools that might have functioned as antibiotic activity (Tamas and Huttova, 1996 and Paolo and Ester, 2000).

The other inducer exhibit moderate peroxidase activity ranging from 1.50: 0.72 of absorbance at 425. However, most of the remaining inducers exhibit values ranging from 0.002-0.015 of polyphenol oxidase activity at absorbance 495.

As for sulfur it indicates high activity of peroxidase 1.62 and ranked the third among the examined inducers of polyphenol oxidase being 0.016 absorbances at 495.

Thus, potassium bicarbonate might affect the production of both of PO and PPO resulting in an inhibition effects through changing the hydrogen ion concentration of the surrounding medium that might restrict the infection with the causal pathogen (Crisp *et al.* 2006 and Sawant and Sawant 2008).

Salicylic acid from another hand might affect as it

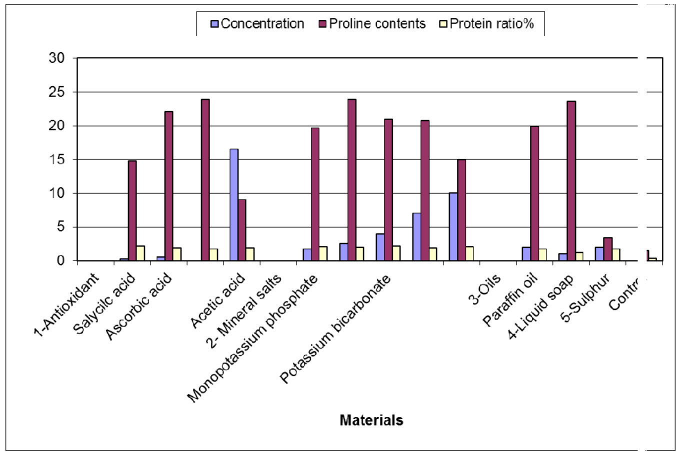

Fig. 3: Changes in proline contents and protein ratio as result of eco-friendly application.

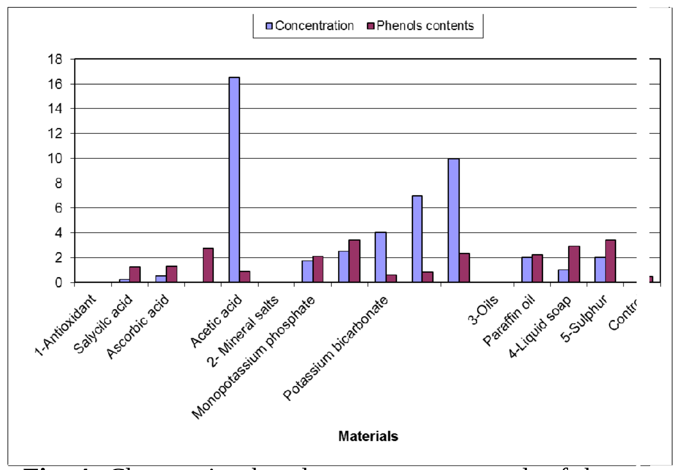

Fig. 4: Changes in phenols contents as a result of the eco-friendly application.

considered the exogenous factor that triggers the systemic signal transduction pathway in several plant cultivars through induction oxidative enzymes, phytoalexins and accumulation of pathogenesis-related proteins, some of which possess antimicrobial properties. Also, salicylic acid might have an impact on plant growth development. Also, it might regulate such enzyme activities which are the major components of induced plant defense mechanisms against several stresses (Tamas and Huttova, 1996).

Sulfur resulted in high values of both PO and PPO. The treated plants exhibit lower disease severity compared with the other inducers. This might be also due to that sulfur is considered as a preventive agent through competing for oxygen resulting in hyphal and conidiophores malformation and shrinkage for the pathogen growth (Singh and Varma, 2002).

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