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Evaluation of SAR chemicals under field conditions for the management of leaf spot (*Phloeospora maculans*) disease in mulberry

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Abstract

Effect of various chemicals namely Isonicotinic acid, Calcium chloride, Ascorbic acid, Ethylene diamine tetra acetic acid etc., at different concentrations on the management of leaf spot disease in mulberry caused by *Phloeospora maculans* was studied. The findings of the study showed that minimum disease incidence and intensity with maximum per cent disease control was observed in BABA (2.0 mg/ml) followed by carbendazim (0.5 mg/ml), INA (2.0 mg/ml) and salicylic acid (1.5 mg/ml).

Keywords: mulberry, disease, leaf spot, field evulation, chemicals

Introduction

Mulberry (Morus sp.) is a perennial tree or shrub used as a food source for the domesticated silkworm, Bombyx mori. In J&K, the sericulture industry is an important enterprise that is increasingly being perceived as a promising alternative source of income generation for rural small-scale farmers. However, diseases are some of the limiting factors for successful mulberry cultivation. Like other plants, mulberry is affected by a number of diseases caused by leaf fungi, bacteria, viruses and nematodes. The foliar diseases are more important than the diseases affecting the other plant parts, as these have direct relation to accessibility of mulberry leaves due to air- borne nature of pathogens. The quality and quantity of mulberry leaves is affected by various kinds of diseases like leaf spot, leaf rust, powdery mildew, leaf blight, twig blight, violet root rot, white root rot etc. Among these leaf spot is most prevalent disease. The leaf spot disease not only effect the quantity of mulberry leaves, but also their nutritive value. When leaf spot affected leaves are fed to the silkworm larvae, it results in poor larval growth, cocoon crop and affects the commercial characters of cocoons. The week larvae also become more susceptible to diseases, thereby often resulting in drastic reduction in cocoon yield (Sikdar et al., 1979; Qadri et al., 1999) ^[3, 4]. The leaf spot disease is very common in Kashmir valley due to favourbale environmental conditions (temperature 20-30°C and humidity 70-75%) for disease development. It appears from early May and reaches to its peak in the month of July, August and September. The disease incidence and intensity was recorded 41.44 and 24.44 per cent, respectively in the year 1999, with all the genotypes maintained in the germplasm bank of the institute affected by this disease (Kausar, 2005)^[5]. Foliar sprays with carbendazim 50 WP @ 0.05% and Captan 50 WP @ 0.4% were found most effective fungicide for controlling leaf spot (Munshi et al., 1987; Ahsan et al., 1990; Ganga and Chetty, 1996) ^[6, 7, 8]. Triazoles 500 ppm (hexaconazol, penconazole and bitertanol) were also found more effective than carbendazim (Tanki et al., 2005)^[9]. Although chemical measures have been suggested for the control of disease in tropical conditions (Philip et al., 1994; Gupta; 2001) ^[10, 11], the chemical fungicides have not gained wide acceptance among the sericulturists owing to their high cost, the possible toxicity to silkworms, potential health hazards to mankind and environmental imbalance (Govindaiah et al., 1996)^[12]. The fungicides besides causing the environmental hazards, adversely affects the non-target species including beneficial organisms and thereby disturbing the ecological balance. Moreover these chemicals are site specific in their action and provide protection only for a short period. Therefore, the frequent application of these fungicides are required for successful disease control which leads to the development of resistance in pathogen against these fungicides and thus either higher doses of recommended chemical or an effective alternative non- toxic chemicals are required. In addition to this, prolonged and extensive use of fungicides especially carbendazim results in the development of resistance, which is now an established fact (Singh, 1991)^[13].

Moreover, these chemicals are unable to reduce crop loss in a situation, where numbers of pathogens are involved and their incidence is frequent in nature.

Due to all these constraints and problems associated with the chemical control, it is necessary to find out the alternatives of chemical control measures by developing an ecologically safe method for protecting the mulberry plants against the pathogens.

A variety of constitutive barriers (physical and chemical), which are present in plant prior to infection are collectively responsible for the natural resistance of plants. Plant defense system activates these barriers upon recognition of a pathogen or its products. The disease occurs either from failure of this recognition event or the ability of pathogen to avoid or overcome the resistance response.

When a chemical or biological agent induces or activates the defense mechanism for the production or accumulation of defense components in the host plant, it may be regarded as Induced Systemic Resistance (ISR) or Systemic Acquired Resistance (SAR). In the recent past, the research on SAR chemicals carried out on many plant-pathogen systems revealed that there are various non-toxic chemicals that elicit the Systemic Acquired Resistance in plants (Lyon *et al.*, 1995; Ebel and Mithofer, 1998; Purkayastha, 1998;

Vidhyasekaran, 1998; Oostendrop *et al.*, 2001) ^[1, 2, 14-17]. Therefore a potential disease management strategy, which can be an alternative to chemical control, would be requested to activate the plant defense system by using non-toxic chemicals. Keeping in view, the present experiment was carried out to evaluate SAR chemicals under different concentration at field condition for the management of Leaf Spot (*Phloeospora maculans*) in mulberry

Materials and Methods Disease management

For the management of leaf spot disease of mulberry (*Morus* spp.) different systemic acquired resistance inducers were shortlisted and were evaluated under field conditions to test the efficacy of the SAR chemicals at commercial level.

Studies under greenhouse conditions

Experiment was conducted in greenhouse to test the efficacy of below mentioned systemic acquired resistance inducers at three different concentrations:

S. No.	Systemic acquired resistance inducer	Concentration (mg/ml)		
1.	Salicylic acid	0.5	1.0	1.5
2.	Isonicotinic acid	1.0	1.5	2.0
3.	Calcium chloride	5.0	10.0	15.0
4.	Ascorbic acid	1.0	2.0	3.0
5.	Ethylene diamine tetra acetic acid	0.25	0.50	1.0
6.	Sodium salicylate	0.10	0.15	0.20
7.	β-amino butyric acid	1.0	1.5	2.0
8.	Check (carbendazim) 50% WP	0.5	0.5	0.5
9.	Control (distilled water sprayed leaves)	-	-	-

The experimental trial was laid on one year old sapling of Kokuso-27 susceptible variety of mulberry planted in poly bags and kept in greenhouse as per the completely randomized design (CRD) during the year 2011 and 2012. All the seven systemic acquired resistance inducers (SAR) were tested at three concentrations, each concentration was replicated thrice and each replication comprised of three plants.

Each chemical was dissolved in distilled water to make different concentrations (mg/ml) and was applied individually to mulberry plants by foliar spray on both the sides of leaf, one week after the first spray, leaves were inoculated with the fungal spores of the freshly isolated pathogen *Phloeospora maculans*. High humidity and optimum temperate 25 ± 1 °C was maintained inside the greenhouse. The spore concentration was adjusted 30-40 spores per field (10x X 10x); one week after inoculum second spray of chemical is done. The elicitation of systemic acquired resistance of leaf spot disease was monitored 45 and 70 days after sprouting by visually estimating the leaf spot symptom. The total number of leaves on a plant was counted, and then diseased leaves were categorized in six grades on the basis of number of spots by adopting the scale (Plate 1) given by Croxall *et al.* (1952) with slight modification as per the requirement as follows:

Grade	Leaf area affected
0	Leaves free from infection
1	1-5 spots
2	6-10 spots
3	11-15 spots
4	16-20 spots
5	Above 21- coalesces

The effectiveness of various systemic acquired resistance inducing chemicals at different concentrations was evaluated by recording the per cent disease incidence, per cent disease intensity and per cent disease control by using the following formula's:

Per cent disease incidence =
$$\frac{\text{No. of diseased leaves}}{\text{Total No. of leaves examined}} \times 100$$

Per cent disease intensity =
$$\frac{\sum \text{ numerical values x Grades}}{\text{Total No. of leaves examined}} \times \frac{100}{\text{Max. Grade}}$$



Plate 1: Scale used for measurement of disease intensity

Grade 3 = 11-15 spots Grade 4 = 16-20 spots Grade 5 = above 21 coalesces

Per cent disease control = $\frac{\text{C-T}}{\text{C}} \times 100$

C = Per cent disease intensity in controlT = Per cent disease intensity in treatment

Results and Discussion

Highest disease incidence was observed in EDTA (1 mg/ml) 25.16 per cent disease incidence and again observed to be least effective in field.

Per cent disease control 45 days after pruning

Per cent disease control 45 days after pruning ranged (Table 1; Fig. 1) from 26.08 to 69.24 per cent. Highest per cent disease control was found in BABA (2.0 mg/ml) 69.24 per cent followed by carbendazium (0.5 mg/ml), INA (2.0 mg/ml), salicylic acid (1.5 mg/ml), sodium salicylate (2.0 mg/ml), calcium chloride (10 mg/ml) and EDTA (1.0 mg/ml) with per cent disease control 66.48, 49.61, 48.64, 45.53, 40.24, 36.25 and 26.08 per cent, respectively.

Disease incidence 70 days after pruning

Per cent disease incidence was higher at 70 days after pruning as compared to 45 days pruning. It ranged (Table 8; Fig. 2) from 13.05 to 51.32 per cent. Least per cent disease incidence was observed in BABA (2.0 mg/ml) 13.05 per cent followed by carbendazium (0.5 mg/ml) 13.97 per cent, INA (2.0 mg/ml) 22.63 per cent, salicylic acid (1.5 mg/ml) 24.53 per cent, sodium salicylate (2.0 mg/ml) 25.82 per cent, calcium chloride (10 mg/ml) 28.56 per cent, ascorbic acid (3.0 mg/ml) 28.37 per cent and was observed highest in EDTA (1.0 mg/ml) 29.99 per cent.

Per cent disease control 70 days after pruning

Per cent disease control at 70 days after pruning ranged (Table 2; Fig. 2) from 75.57 to 41.56 per cent. Highest per cent disease control was observed in BABA (2.0 mg/ml) with 74.57 per cent, followed by carbendazim (0.5 mg/ml) with 72.77 per cent, INA (2.0 mg/ml) with 55.90 per cent, salicylic acid (1.5 mg/ml) with 52.20 per cent, sodium salicylate (2.0 mg/ml) with 49.68 per cent, calcium chloride (10 mg/ml) with 44.71 per cent, ascorbic acid (3.0 mg/ml) with 44.34 per cent and least per cent disease control was found in EDTA (1.0 mg/ml) with 41.56 per cent disease control.

 Table 1: Effect of SAR chemicals on per cent disease incidence and per cent disease control (45 days after pruning) under field conditions

Treatment		Conc.	45 days after pruning				
Treatment code	Chemical	(mg/ml)	2011	2012	Pooled	Per cent disease control	
T1	Salicylic acid	1.5	16.54 (4.18)	18.43 (4.40)	17.48 (4.29) ^c	48.64	
T ₂	Isonicotinic acid	2.0	16.84 (4.22)	17.45 (4.29)	17.15 (4.25) ^c	49.61	
T3	Calcium chloride	10	19.21 (4.49)	21.48 (4.74)	20.34 (4.61) ^e	40.24	
T 4	Ascorbic acid	3.0	19.65 (4.54)	23.75 (4.97)	21.70 (4.75) ^f	36.25	
T5	Ethylene diamine tetra acetic acid	1.0	23.80 (4.98)	26.52 (5.24)	25.16 (5.11) ^g	26.08	
T ₆	Sodium salicylate	2.0	17.73 (4.32)	19.36 (4.51)	18.54 (4.41) ^d	45.53	
T7	β-amino butyric acid	2.0	9.35 (3.21)	11.60 (3.54)	10.47 (3.38) ^a	69.24	
T ₈	Check (Carbendazim 50% WP)	0.5	10.26 (3.35)	12.56 (3.68)	11.41 (3.51) ^b	66.48	
T9	Control (Distilled water sprayed leaves)	-	32.56 (5.79)	35.51 (6.04)	34.04 (5.91) ^h	-	
	CD (p ≤ 0.05)		0.201	0.149	0.120		

*Figures in parenthesis are square root transformed values

**Figures superscripted with identical letter(s) do not differ significantly

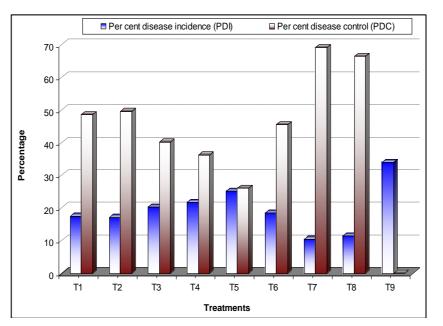


Fig 1: Effect of SAR chemicals on per cent disease incidence and per cent disease control (45 days after pruning) under field conditions T_1 = Salicylic acid; T_2 = Isonicotinic acid; T_3 = Calcium chloride; T_4 = Ascorbic acid; T_5 = Ethylene diamine tetra acetic acid; T_6 = Sodium salicylate; T_7 = β -amino butyric acid; T_8 = Check (Carbendazim 50% WP); T9 = Control (Distilled water sprayed leaves)

Table 2: Effect of SAR chemicals on per cent disease incidence and per cent disease control (70 days after pruning) under field conditions

Treatment		Conc.	Conc. 70 days after pruning				
Treatment code	Chemical	(mg/ml)	2011	2012	Pooled	Per cent disease control	
T_1	Salicylic acid	1.5	23.55 (4.95)	25.52 (5.14)	24.53 (5.05) ^d	52.20	
T_2	Isonicotinic acid	2.0	21.68 (4.76)	23.58 (4.95)	22.63 (4.86) ^c	55.90	
T ₃	Calcium chloride	10	27.46 (5.33)	29.65 (5.53)	28.37 (5.41) ^e	44.71	
T_4	Ascorbic acid	3.0	27.48 (5.33)	29.26 (5.50)	28.56 (5.43) ^f	44.34	
T5	Ethylene diamine tetra acetic acid	1.0	28.54 (5.43)	31.43 (5.69)	29.99 (5.56) ^h	41.56	
T_6	Sodium salicylate	2.0	25.46 (5.14)	26.19 (5.21)	25.82 (5.17) ^g	49.68	
T_7	β-amino butyric acid	2.0	12.47 (3.66)	13.63 (3.82)	13.05 (3.74) ^a	74.57	
T8	Check (Carbendazim 50% WP)	0.5	13.41 (3.79)	14.52 (3.93)	13.97 (3.86) ^b	72.77	
T 9	Control (Distilled water sprayed leaves)	-	48.52 (7.03)	54.11 (7.42)	51.32 (7.23) ^I		
	CD (p ≤ 0.05)		0.110	0.142	0.086		

*Figures in parenthesis are square root transformed values

**Figures superscripted with identical letter(s) do not differ significantly

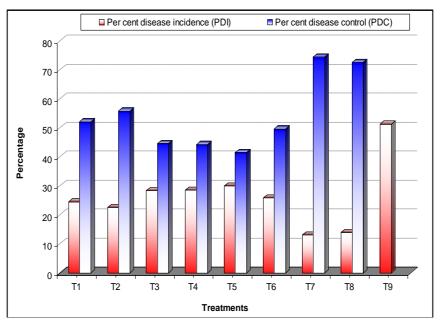


Fig 2: Effect of SAR chemicals on per cent disease incidence and per cent disease control (70 days after pruning) under field conditions T₁ = Salicylic acid; T₂ = Isonicotinic acid; T₃ = Calcium chloride; T₄ = Ascorbic acid; T₅ = Ethylene diamine tetra acetic acid; T₆ = Sodium salicylate; T₇ = β-amino butyric acid; T₈ = Check (Carbendazim 50% WP); T₉ = Control (Distilled water sprayed leaves)

Efficacy of SAR chemicals on per cent disease intensity under field conditions

Per cent disease intensity after 45 and 70 days of pruning (Table 3 and 4), under field conditions, revealed that all the treatments significantly lowered as compared to control.

Disease intensity 45 days after pruning

Perusal of data (Table 3; Fig. 3) revealed that per cent disease intensity after 45 days of pruning ranged from 7.05 to 28.03 per cent in chemicals (SAR activators) treatments in comparison to control 28.03 per cent indicating that all the SAR chemicals along with check (fungicide carbendazim) are significantly effective in lowering the disease intensity. The least disease intensity after 45 days of pruning was found in BABA (2.0 mg/ml) with 7.05 per cent followed by carbendazim (0.5 mg/ml) 7.91 per cent and INA (2.0 mg/ml) 13.74 per cent; whereas, salicylic acid (1.5 mg/ml), sodium salicylate (2.0 mg/ml) and calcium chloride (10 mg/ml) were at par with 14.81, 15.23 and 15.81 per cent disease incidence respectively. They were followed by ascorbic acid (3.0 mg/ml) 17.61 per cent disease incidence. Highest disease intensity was observed in EDTA (1 mg/ml) 19.60 per cent.

Disease control 45 days after pruning

Per cent disease control after 45 days of pruning (Table 3; Fig. 3) ranged from 74.84 to 30.07 per cent. Highest per cent disease control was observed in BABA (2.0 mg/ml) with 74.84 per cent followed by carbendazim (0.5 mg/ml) 71.78 per cent, INA (2.0 mg/ml) 50.98 per cent, salicylic acid (1.5 mg/ml), 47.16 per cent, sodium salicylate (2.0 mg/ml) 43.59 per cent, ascorbic acid (3.0 mg/ml) 38.77 per cent and EDTA (1.0 mg/ml) 30.07 per cent with least per cent disease control.

Table 3: Effect of SAR chemicals on per cent disease intensity and per cent disease control (45 days after pruning) under field conditions

	Conc.	45 days after pruning				
Treatment code	Chemical	(mg/ml)	2011	2012	Pooled	Per cent disease control
T1	Salicylic acid	1.5	12.52 (3.67)	17.10 (4.25)	14.81 (3.96) ^d	47.16
T2	Isonicotinic acid	2.0	13.01 (3.73)	14.48 (3.92	13.74 (3.83) ^c	50.98
T3	Calcium chloride	10	13.58 (3.81)	18.04 (4.36)	15.81 (4.08) ^d	43.59
T 4	Ascorbic acid	3.0	15.52 (4.06)	18.81 (4.44)	17.61 (4.25) ^e	38.77
T5	Ethylene diamine tetra acetic acid	1.0	18.52 (4.41)	20.68 (4.65)	19.60 (4.53) ^f	30.07
T6	Sodium salicylate	2.0	14.38 (3.91)	16.08 (4.13)	15.23 (4.02) ^d	45.66
T ₇	β-amino butyric acid	2.0	6.73 (2.77)	7.37 (2.89)	7.05 (2.83) ^a	74.84
T8	Check (Carbendazim 50% WP)	0.5	7.33 (2.88)	8.48 (3.07)	7.91 (2.97) ^b	71.78
T9	Control (Distilled water sprayed leaves)	-	26.57 (5.25)	29.48 (5.52)	28.03 (5.38) ^g	-
	CD (p ≤ 0.05)		0.201	0.165	0.125	

*Figures in parenthesis are square root transformed values

**Figures superscripted with identical letter(s) do not differ significantly

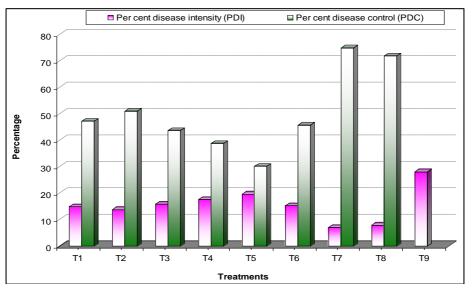


Fig 3: Effect of SAR chemicals on per cent disease intensity and per cent disease control (45 days after pruning) under field conditions $T_1 =$ Salicylic acid; $T_2 =$ Isonicotinic acid; $T_3 =$ Calcium chloride; $T_4 =$ Ascorbic acid; $T_5 =$ Ethylene diamine tetra acetic acid; $T_6 =$ Sodium salicylate; $T_7 = \beta$ -amino butyric acid; $T_8 =$ Check (Carbendazim 50% WP); $T_9 =$ Control (Distilled water sprayed leaves)

Disease intensity 70 days after pruning

Disease intensity was higher after 70 days of pruning as compared to 45 days after pruning. It ranged (Table 10; Fig. 4) from 9.21 to 36.99 per cent. Least disease intensity was observed in BABA (2.0 mg/ml) with 9.21 per cent which is at par with carbendazim (0.5 mg/ml) with 9.29 per cent disease intensity. They were followed by INA (2.0 mg/ml) and salicylic acid (1.5 mg/ml) with disease intensity 15.53 and 16.0 per cent were at par with each other, followed by sodium

salicylate (2.0 mg/ml) 17.23 per cent, calcium chloride (10 mg/ml) 20.57 per cent and ascorbic acid (3.0 mg/ml) were at par. Highest disease intensity was observed in EDTA (10 mg/ml) 21.63 per cent and observed to be least effective among all the treatments.

Disease control 70 days after pruning

Per cent disease control after 70 days of pruning (Table 4; Fig. 4) ranged from 41.52 to 75.10 per cent. Highest disease

control was observed in BABA (2.0 mg/ml) with 75.10 per cent followed by carbendazium (0.5 mg/ml) 74.88 per cent, INA (2.0 mg/ml) 58.01 per cent, salicylic acid (1.5 mg/ml) 56.50 per cent, sodium salicylate (2.0 mg/ml) 53.41 per cent, calcium chloride (10 mg/ml) 44.39 per cent, ascorbic acid (3.0 mg/ml) 43.33 per cent and least per cent disease control was observed in EDTA (1.0 mg/ml) 41.52 per cent disease control.

Disease management

There are some chemicals which are not fungi toxic but induce resistance in plants systemically against the test pathogens and thus help ward off infection. Such chemicals often referred to as systemic acquired resistance (SAR) inducers have been exploited for the control of many plant diseases of economic importance (Hammerschmidt, 1999; Murphy et al., 2000; Nanda Kumar et al., 2001; Niranjana et al., 2003; Raupach and Kloepper, 1998 and 2000; Van loon et al., 2006; Andreu, 2006). The use of SAR inducers is gaining importance day by day owing to their eco-friendly nature with no adverse effects on human health and ecology (Mur et al., 2000). Foliar spray with SAR chemicals in the present study revealed that all the SAR chemicals along with fungicide carbendazim have significantly reduced disease incidence and intensity under green house and field conditions. Under green house conditions all the chemicals behave most effectively at their higher concentrations. Disease incidence ranged from 12.59 to 51.97 per cent at 45 days after sprouting and 13.21 to 64.78 per cent at 70 days after sprouting. Disease intensity ranged from 10.48 to 42.30 per cent at 45 days after sprouting and 12.70 to 51.52 per cent at 70 days after sprouting. Minimum disease incidence and intensity with maximum per cent disease control was observed in BABA (2.0 mg/ml) followed by carbendazim (0.5 mg/ml), INA (2.0 mg/ml) and salicylic acid (1.5 mg/ml).

Similar results were obtained under field conditions. Disease incidence ranged from 10.47 to 34.04 per cent at 45 days after sprouting and 13.05 to 51.32 per cent at 70 days after sprouting. Disease intensity ranged from 7.05 to 28.03 per cent at 45 days after sprouting and 9.29 to 36.99 per cent at 70 days after sprouting. Minimum disease incidence and intensity with maximum per cent disease control was observed in BABA (2.0 mg/ml) followed by carbendazim (0.5 mg/ml), INA (2.0mg/ml) and salicylic acid (1.5 mg/ml). Among all elicitors tested, 4-Amino-n-butyric induced greater systemic resistance in mulberry. These results are in agreement with Zhang et al. (2001) who reported the significant reduction in leaf spot of peanut. Similar results were also obtained by Cohen et al. (1994) in protection of tomato plants against Phytopthora infestans, and Papavizas (1968) in pea against Aphanomyces euteiches. Jeun et al. (2000) also reported that DL-3-amino butyric acid could induced systemic acquired resistance in tomato plants against Phytophthora infestans. This may be because of ability of 4-Amino-n-butyric acid to increase the content of signal molecule, salicylic acid (SA) in plant leaves, which induce systemic resistance in plants. Colson et al. (2000) also reported that INA and BTH reduced the susceptibility of cotton plants against leaf spot (Alternaria macrospora), bacterial blight (Xanthomonas campestris Pv. malvacearum) and wilt (Verticillium dahliae). These observations are consistent with the earlier reports which demonstrate the induction of systemic resistance by exogenous application of various PGPR strains and chemical elicitors to plants against a range of fungal, bacterial and viral pathogens (Dempsey and Klessig, 1994; Hammerschmidt and Kuc, 1995; Lyon et al.,

1995; Klessig *et al.*, 2000; Gupta *et al.*, 2004) ^[1]. Munshi *et al.* (1987) ^[1, 6] and Siddaramiah and Hedge (1989) have also recommended effective control of carbendazim @ 500 ppm and 1000 ppm. These results were also supported by Gupta *et al.* (2008) who also reported that BABA is most effective against leaf spot and leaf rust of mulberry among the SAR chemicals tested.

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