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In vitro efficacy of different chemicals and bio-products against *Xanthomonas campestris* pv. *mangiferaeindicae* causing bacterial leaf spot of mango

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ABSTRACT

Mango is unique in its taste known as the king of the all fruits. Bacterial leaf spot is devastating disease significantly lowers the mango yield. Four chemicals (Score, Bowek, Success and Ridomel Gold) and bio-products (Plant Protector, Biosal, Vampire and Rigorous) efficacy at 1%, 2% and 3% doses were evaluated after 12, 24, 48 and 72 h by using the inhibition zone technique against *Xanthomonas campestris* pv. *mangiferaeindicae*. Among different chemicals, at 3 % concentration, Ridomil-Gold was more significant inhibited 1.35 cm bacterial colony growth. Plant Protector was most significant (1.15 cm) bio-product inhibiting bacterial colony growth at 3% after 72 h interval.

KEYWORDS: Mango (*Mangifera indica* L), Bacterial Leaf Spot of Mango, *Xanthomonas campestris* pv. *mangiferaeindicae*, Bio-products, Ridomil-Gold

INTRODUCTION

Mango (*Mangifera indica* L) is a delicious stone fruit known as "The King" of all fruits. It belongs to family Anacardiace (Juliano and Cuevas, 1932) extensively found in the tropical regions of the world [4].

Pakistan is the fifth largest exporter of mango after the India, China, Thailand and Mexico [1] exports 367 thousand tones with the earning of 9783 million rupees. In Pakistan, mango is cultivated on an area of 652 thousand hectares with annual production of 1732 thousand tones shares 6% in world's mango production.

More than one hundred and fourty diseases in the world significantly reduces mango yield and quality which results into poor earning of formers [14]. Bacterial leaf spot of mango caused by *Xanthomonas campestris* pv. *mangiferaeindicae* is a devastating disease of mango in all over the world [3] which results in 10-70% fruit drop, 10-85% in yield loss and 5-100% losses in storage [16].

Chemicals are cheap and effective tools for disease management in some aspects [7]. Non judicious chemicals applications aggressively damaging biotic and abiotic environments [10]. Having toxic residual effects, these lethal compounds enters in food web and store in adipose tissues of humans and of animals results in major organs failure. Despite having limitations, biological control is unique and effective where nature

remains in equilibrium. Toxicological threats have been minimized where organisms are introduced against organisms [17].

Evaluating different chemicals and bioproducts in field against pathogen needs more economic resources [12]. It is better to assess the efficacy of chemicals and bio-products against the pathogen firstly in the laboratory and then the most significant one is evaluated in filed conditions, this minimizes the experimental cast. Present research was coined to evaluate the efficacy of different chemicals and bio-products against the colony diameter of *Xanthomonas campestris* pv. *mangiferaeindicae* in lab conditions using the inhibition zone technique.

MATERIALS AND METHODS

Disease samples were taken from infected tress of University of Agriculture, Faisalabad, Pakistan. and were preserved at 4 ^{o}C for further study. Diseased tissues were surface sterilized in 0.1% $HgCl_2$ for two minutes then rinsed twice with distilled water and air dried. Disease tissues were macerated in distilled water and resultant was filtered by Watman No.1filter paper. Three successive dilutions (10^{-1}, 10^{-2} and 10^{-3}) were made and the bacterium was isolated by dilution plate technique. Modified Nutrient Glucose medium (glucose= 5g/L and nutrient agar = 15g/L) was used for bacterial colony isolation. Plates were wrapped and incubated for 48 hours at 25 ^{o}C. Recovered colonies were purified by streaking method and were preserved (50% glycerin solution) for further study at 4 ^{o}C.

Freshly growing aqueous bacterial culture was prepared in nutrient broth and mixed in sterilized Nutrient Glucose agar medium as was poured in petri plates. After solidification, wells of 1 cm were made at the center by cork borer. Four chemicals (Score, Bowek, Success and Ridomel Gold) and bio-products (Protector, Biosal, Vampire and Rigorous) efficacy at 1%, 2% and 3% doses was evaluated by using the inhibition zone technique. In control, sterile water was poured. Treated plates were wrapped and incubated at 25 ^{o}C. Data was recorded by measuring the inhibition zones area with an ordinary ruler in centimeter scale after 12, 24, 36, 48 and 72 h of subsequent intervals. Experiment was conducted in Completely Randomized Design (CRD) with three replications.

Statistical Analysis:

Recorded data were analyzed through Analysis of Variance (ANOVA) and treatments means were compared by Fisher's Least Significant Difference (LSD) test. Data was processed statistically through SAS (9.3) software and was represented by Microsoft Excel (2007).

RESULTS AND DISCUSSION

A significant difference ($p \leq 0.05$) was observed for efficacy of four different chemicals used against colony growth of *Xanthomonas campestris* pv. *mangiferaindica*. Inhibition zone's area by different tested chemicals significantly increased with respect to increase in dose and time of treatment. After 12 h of treatment, none of the four chemicals were found effective at 1% concentration. Ridomil-Gold at 2% and 3% was the only one to inhibit colony growth (0.20 cm) as compared to others. After 24 h, Score was the most significant inhibited (0.30 cm) colony diameter at 1% concentration, no significant difference was observed between Ridomil-Gold and Success. A similar trend was noted at 2% concentration. Score was found to be most significant (0.60 cm) at 3% dose followed by Ridomil-Gold (0.58 cm). After 36 h, significant results were noted by Ridomil-Gold compared to other treatments at 1 %, 2% and 3% concentrations. No inhibition zones were recorded in the control treatment. At 3% concentration, Bowak was the least significant (0.60 cm) to inhibit bacterial colony growth. After 48 hours of treatment, significant efficacy was resulted by Ridomil-Gold at 1%, 2% & and 3% concentrations inhibited 0.62 cm, 0.82 cm and 0.90 cm zones. No significant difference was seen between Bowak and Score treatments at 1% concentration. At 2%, Score (0.60 cm) was significant to Bowek (0.55 cm) and Success (0.55 cm) but was least significant as compared to Ridomil-Gold (0.82 cm). No significant difference in efficacy of Bowak and Score treatments was observed. Bowak was found to be least significant as compared to Ridomil-Gold, Success and score while in control treatment no inhibition zone was recorded at 3% dose. After 72 h, significant treatment efficacy was noted by Ridomil-Gold at all the concentrations. At 3 % concentration, Ridomil-Gold inhibited 1.35 cm bacterial colony growth was more significant than other treatments. Score was least significant to Ridomil-Gold but was significant as compared to Bowak and Score. Bowek was least significant to inhibit bacterial colony growth of *Xanthomonas campestris* pv. *mangiferaindica* after 72 hours at 3% concentration.

The efficacy of different bio-products significantly increased with the increase of used concentration. Significant increase in the inhibition zone area was noticed as time passed after pouring bio-products in bacterial cultured plates wells. Analysis of Variance revealed a significant interaction between time, concentration and treatments. After 12 h at 1% concentration, no inhibition zone was observed by any of the product. Plant

Protector was the only one to inhibit bacterial growth at 2% and 3% concentrations. After 24 h, Plant Protector was the most significant (0.15 cm) to inhibit bacterial colony growth comparing to other products at 1% concentration. No inhibition zone was seen by Biosal product. Vampire was less significant (0.10 cm) comparing to Plant Protector (0.15 cm) was more with respect to Rigorous (0.07 cm) Biosal (0.00 cm) and Control (0.00 cm) treatments. At 2% and 3%, a significant increase in the inhibition zone was observed with respect to 1% concentration. Plant Protector and Rigorous were significantly same in efficacy to inhibit bacterial colony growth at 2% and 3% concentrations. No significant difference in efficacy of Biosal and Vampire products was recorded. After 36 h at 1% concentration, Rigorous (0.15 cm) was the least significant to inhibit bacterial colony growth. Plant Protector was the most significant product to inhibit bacterial colony. At 2%, no significant difference in bactericidal efficacy of Plant Protector (0.40 cm), Rigorous (0.40 cm) and Biosal (0.40 cm) was measured. At 3% concentration, Plant Protector (0.52 cm) was the most significant as compared to other treatments used.

Relative to 24 h, significant increase in inhibition zones area was measured after 48 hours at 1% concentration. No significant difference in bacterial efficacy of Biosal (0.34 cm) and Rigorous (0.35 cm) was measured. At 2% and 3% Plant Protector was most significant to impede bacterial multiplication. At 3% dose, Biosal and Vampire were significantly same in efficacy to inhibit colony radii.

After 72 h, Plant Protector was the most significant at 1%, 2% and 3% concentrations as compared to other treatments. No significant difference in bacterial zone was recorded by Biosal and Rigorous treatments. At 2 %, Biosal was found least significant (0.50 cm) with respect to others bio-products (Plant Protector, Vampire and Rigorous) except Control (0.00 cm). At 3%, Vampire (0.85 cm) and Rigours (0.85 cm) were significantly same in efficacy. Plant Protector was the most significant to inhibit *Xanthomonas campestris* pv. *mangiferaeindicae* colony growth (Table 4).

For qualitative comparison of different product's efficacies, inhibition zone technique is the most significant especially against bacterial pathogens [18]. Despite simple and easy, results can be estimated through visual comparison. Indeed minimizing experimental cost to evaluate test treatments, it's a cheap and time saving way for evaluation.

Criticizing inhibition zone technique, agar porousity varies upto 30 daltons [5]. Poured fluid in the well at the center of the plate diffuses all around and inhibits bacterial colony growth. Adding more agar powder in culture media synthesis effects directly diffusion rate. More porousity facilitates more diffusion of fluids. Viscosity and solubility of fluids impact inhibition zone. If tested fluid is more viscous regarding how much is effective, the inhibition zone area will be small due to poor diffusion [11]. Similarly, solubility greatly reflects the chemical's efficacy and diffusion rate. More the solubility more the diffusion rate will. Chemical stability directly influence inhibition zone area. Low volatile chemicals evaporates slowly and remained contacted with test pathogen for longer time.

Inhibition zone technique doesn't reflect the exact tested fluid efficacy. In field, environment fluctuates considerably and may alter the stability of chemical. Elevated air temperature events dissociates bonds of applied chemical and also effects pathogen growth; ultimately doesn't represents the exact compound's efficacy against a particular pathogen. Prevailing atmospheric humid conditions cognitively influences evaporation rate. In low humidity, significant increase in evaporation rate directly reduces applied fungicide molecules on the leaf surface if applied foliar. To increase the applied compound efficacy, adhesives are added as inert materials to bind the chemicals on leaf surface. More the time of attachment, more the efficacy will. In early morning, dew droplets favors fungal adhesion on leaf surface [6]. Pathogenic bacterial entrance in plants prompted as stomata remains mostly close due to high air humidity in morning and evening hours. Contact bactericides if applied in morning hours is more effective due to low evaporation losses and stomatal entry. Systemic chemotherapuents especially having basipetal mode of action are more effective when applied in sunny conditions which grace stomatal opening [8].

Chemicals used in present study generally having fungicidal property, present study clearly describes they have bactericidal effects as well. Ridomil – Gold was found most significant to inhibit bacterial colony growth. It chiefly contains Metalaxl-M which inhibits RNA synthesis in fungal cell but not confirmed how it inhibits bacterial life activity.

Thirumalesh et al. [19]evaluated different treatments (Copper oxychloride + copper sulphate; streptocycline + bactrinashak; mancozeb + copper oxychloride, mancozeb + bavistin and bavistin + bactrinashak,) against bacterial black spot of mango. Treatments including copper sulphate and copper oxychloride significantly showed inhibition zone, whereas bavistin alone was less effective.

Thirteen antibacterial substances alone (Vancomycin, Amoxycillin, Bactrinashak, Ciprofloxacin, Copper sulphate, Copper oxychloride, Tetracycline, Bacitracin) and in combinations (Ciprofloxacin + Copper sulphate, Ciprofloxacin + Bactrinashak, Ciprofloxacin + Copper oxychloride, Ciprofloxacin + tetracycline and tetracycline + bactrinashak) were evaluated against *Xanthomonas campestris* pv. *Mangiferaeindicae*. Treatments including copper sulphate and copper oxychloride significantly reduced disease symptoms on plants [2]. Biosal, Vampire and Rigorous are natively used bio-pesticides claimed to be homeopathics but is not

known what these biopesticides actively contains and how interfere with bacterial development. Plant Protector (*Potassium acetyl benzoic acid*) significantly retarded *Xanthomonas campestris* pv. *Mangiferaeindicae* growth, research is needed to elaborate mechanism of action of this compound. Rashid et al. [13] evaluated five bio-products (Vampire, Plant Protector, Biosal, Rigorous and Vega Plus) on five gram lines (06001, 03008, 093127, 05007 and 05014) against *Ascochyta* blight. Sajid et al. [15]assessed efficacy of Plant Protector, Agrimycine and Copper oxy chloride at 100ppm, 300ppm and 600ppm concentration after 24, 48 and 72 h using inhibition zone technique against *Xanthomonas campestris* pv. *malvacearum*. Plant Protector was found most significant at 600ppm after 72 h.

Conclusion:

To evaluate the chemical against a pathogenic disease in the field conditions directly is resource consuming practice. It is better to assess their efficacy *in vitro* against a pathogen at optimum condition reduces experimental cost considerably. Present study reveals that among chemicals, Ridomil-Gold at 3% concentration will be a significant option to asses in the field conditions against bacterial blight of mango disease. Similarly, Plant protector at 3% will be significant in field against the disease as compared to other tested bio products.

Table 1: Impact of chemicals against bacterial leaf spot colony growth with respect to different time intervals (ANOVA)

Source	DF	SS	MS	F	P
Time	4	11.6504	2.91261	946.51	0.0000**
Concentration	2	2.5615	1.28074	416.20	0.0000**
Treatments	3	1.0989	0.36631	119.04	0.0000**
Time x Concentration	8	0.4755	0.05944	19.32	0.0000**
Time x Treatments	12	0.3604	0.03003	9.76	0.0000**
Concentration x Treatments	6	0.0535	0.00892	2.90	0.0113*
Time x Concentration x Treatments	24	0.1786	0.00744	2.42	0.0009**
Errors	120	0.3693	0.00308		
Total	179	16.7481			

** = Highly significant *= Significant α = 0.05

Table 2: Efficacy of various chemicals at three different doses against bacterial leaf spot of mango regarding to different time intervals

	Time Interval														
	12 hours			24 hours			36 hours			48 hours			72 hours		
	Concentrations			Concentrations			Concentrations			Concentrations			Concentrations		
	1%	2%	3%	1%	2%	3%	1%	2%	3%	1%	2%	3%	1%	2%	3%
Score	0.00 S	0.00 S	0.00 S	0.30 PQ	0.40 NO	0.60 IJK	0.33 OPQ	0.50 LM	0.70 GH	0.45 MN	0.60 IJK	0.85 DE	0.50 LM	0.70 GH	1.00 B
Bowak	0.00 S	0.00 S	0.00 S	0.25 QR	0.37 NOP	0.50 LM	0.30 PQ	0.40 NO	0.60 IJK	0.33 OPQ	0.55 KL	0.68 GHI	0.45 MN	0.67 GHI	0.88 CDE
Success	0.00 S	0.00 S	0.00 S	0.28 QR	0.45 MN	0.55 KL	0.38 NOP	0.50 LM	0.65 HIJ	0.45 MN	0.55 KL	0.80 EF	0.62 HIJK	0.70 GH	0.95 BC
Ridomil-Gold	0.00 S	0.20 R	0.30 PQ	0.28 QR	0.45 MN	0.58 JKL	0.45 MN	0.65 HIJ	0.75 FG	0.62 HIJK	0.82 DEF	0.90 CD	0.85 DE	0.94 BC	1.35 A
Control	0.00 S	0.00 S	0.00 S	0.00 S	0.00 S	0.00 S	0.00 S	0.00 S	0.00 S	0.00 S	0.00 S	0.00 S	0.00 S	0.00 S	0.00 S

Means values sharing similar letters do not differ significantly
LSD = 0.19

Table 1: Impact of bio-products against bacterial leaf spot colony growth with respect to different time intervals (ANOVA)

Source	DF	SS	MS	F	P
Time	4	10.8255	2.70638	920.88	0.0000**
Concentration	2	1.1808	0.59039	200.89	0.0000**
Treatments	3	0.9284	0.30946	105.30	0.0000**
Time * Concentration	8	0.2418	0.03023	10.29	0.0000**
Time * Treatments	12	0.3558	0.02965	10.09	0.0000**
Concentration * Treatments	6	0.1729	0.02881	9.80	0.0000**
Time * Concentration * Treatments	24	0.0904	0.00377	1.28	0.0911*
Errors	120	0.3527	0.00294		
Total	179	14.1482			

** = Highly Significant
* = Significant
α = 0.05

Table 2: Efficacy of various bio-products at three different doses against bacterial leaf spot of mango regarding to different time intervals

	Time Interval														
	12 hours			24 hours			36 hours			48 hours			72 hours		
	Concentrations			Concentrations			Concentrations			Concentrations			Concentrations		
	1%	2%	3%	1%	2%	3%	1%	2%	3%	1%	2%	3%	1%	2%	3%
Plant Protector	0.00 P	0.10 O	0.15 NO	0.15 NO	0.27 KLM	0.35 IJK	0.28 KLM	0.40 HI	0.52 FG	0.50 FG	0.65 E	0.85 BC	0.78 CD	0.90 B	1.15 A
Biosal	0.00 P	0.00 P	0.00 P	0.00 P	0.15 NO	0.24 LM	0.20 MN	0.40 HI	0.38 HIJ	0.34 IJK	0.38 HIJ	0.50 FG	0.45 GH	0.50 FG	0.70 DE
Vampire	0.00 P	0.00 P	0.00 P	0.10 O	0.15 NO	0.25 LM	0.25 LM	0.30 JKL	0.35 IJK	0.40 HI	0.45 GH	0.50 FG	0.65 E	0.70 DE	0.85 BC
Rigorous	0.00 P	0.00 P	0.00 P	0.07 OP	0.27 KLM	0.35 IJK	0.15 NO	0.40 HI	0.45 GH	0.35 IJK	0.55 F	0.65 E	0.45 GH	0.76 D	0.85 BC
Control	0.00 P	0.00 P	0.00 P	0.00 P	0.00 P	0.00 P	0.00 P	0.00 P	0.00 P	0.00 P	0.00 P	0.00 P	0.00 P	0.00 P	0.00 P

Means values sharing similar letters do not differ significantly
LSD=0.09

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