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COMPARATIVE EFFICACY OF BIO CONTROL AGENT BACILLUS SUBTILIS AND FUNGICIDES AGAINST POWDERY MILDEW OF APPLE

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ABSTRACT

Comparative efficacy of Biocontrol agent Bacillud Subtilis and fungicides was studied against powdery mildow of apple at Hill Fruit Research Sub Station Murree during the year 2012-13 for this purpose mycoparasite based product Taegro (*Bacillus subtilis* strain FZB24) and three fungicides (Sulfex gold 80 WP, Topass 100 EC & Topsin M 72 WP) were tested as preventive and curative treatments at their recommended doses under natural environmental conditions.. In cases of preventive sprays Taegro @ 6 g per litre of water gave 70.27 percent disease control. This product @12 g per liter of water gave disease control upto 78.07 percent. Mean incidence in untreated control treatment remained as 33.22 percent. Disease control in Sulfex Gold 80 WP, Topass 100 EC and Topsin M 72 WP remained as 72.78, 82.96 and 76.88 percent respectively. in case of curative treatments at the time of more than 25 percent disease incidence, Taegro @ 6 g per litre of water gave 37.43 percent disease control and @ 12 g per litre of water, it gave disease control upto 44.52 percent. Mean disease incidence in untreated control treatment was remained as 40.00 persent. Fungicides, Sulfex Gold 80 WP, Topass 100 EC and Topsin M 72 WP, gave disease control to 39.76%, 75.02% and 68.51 percent respectively.

KEYWORDS: Apple; Powdery mildew; *Bacillus Subtilis;* Biocontrol; Fungicides; Chemicals; Pakistan.

INTRODUCTION

Malus domestica (Apple) belongs to family Rosaceae. In Punjab, only the Murree Hills, is the place where apples are grown fruitfully. Powdery mildew has emerged as common disease of apple plants but are generally escaped the consideration of the growers. It was, therefore, desirable to identify and describe symptoms of the disease on apple plants. Apple trees can tolerate

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temperature as low as from 0 ^{o}C to 4 ^{o}C (1, 3). Many fungal pathogens attack agricultural crops and the fungi causing powdery mildew diseases, exemplify themselves most commonly scattered and universal pathogenic microbes (5) and reduce yield as much as 20 to 40 percent (2). Powdery mildew disease of cultivated apple is caused by a well known fungus, *Podosphaera leucotricha* (Ell. and Ev.). Diseased apple trees become retarded due to decrease in photosynthesis and transpiration processes, essential for healthy plant growth (7). Flower buds infection and loss in vitality of the infected plant become the basis of yield loss in apple production (20) and thus served the basis of economic loss by declining tree vigor, less flower buds production and production of reduced quality fruit (12). Young leaves and shoots of the infected plant become powdery white and blighted along with fruit russetting. Heavy mildew infectivity is able to reduce yield from aborted blossoms, reduced blooming and retarded shoot growth. Disease infection starts when there is a lack of moisture availability. Therefore the malady too attack apple plants in dry period (10). Fruit infection is very uncommon (4). The disease pathogen spreads asexually, though a study (20) pointed out that ascospores may be the further source of infection. Primary infection of young leaves usually starts from overwintering mycelium in dormant buds that produce conidia for the secondary infection (2). At present, disease control strategies against apple powdery mildew include, use of varieties having resistance or the use of frequent spray applications of effective fungicides. Due to unavailability of resistant apple varieties and cumulative community distresses about pesticides toxic effect on human health and environment, have forced to substitute methods which are efficient, consistent and environmentally secure (11, 14). Biological control agents (BCAs) are versatile microbes and their mode of action against plant pathogenic microbes is well known. These can be used as a potential source and can be included in integrated management strategies against pathogenic microbes (19, 21).

In the absence of established disease controlling technologies against apple powdery mildew in Pakistan, efforts were made to control the disease by using biocontrol agent instead of toxic fungicides. Therefore, this study was conducted to document the status of comparative efficacy of biocontrol agent *Bacillus subtilis* and the fungicides available for the control of apple powdery mildew.

MATERIALS AND METHODS

This study was conducted at Hill Fruit Research Sub Station Murree during the year 2012-13 .Research studies were conducted during 2013. Eight to

ten years old apple plants of variety (cv. Summer Red) were selected at random and maintained by routine and conventional agronomic practices without spraying. There were three plants for each treatment. Pathological investigations were made in Disease Diagnostic Laboratory,. For this purpose, typically infected leaves, shoots, flowers and fruits showing white powdery material were collected for pathogen identity; diseased samples were surface sterilized with 1% Clorox. Small pieces of diseased plant tissues /flowers/ panicles were teased, stained and the pathogen was examined and identified microscopically in an Olympus Microscope Model BX50F-3 with ocular lens of 10 X and objective lens of 100 X (Total magnification of 1000 X) as the methodology described by some workers (2, 10, 23). Twenty inflorescence/panicles were tagged randomly on each apple plant before spraying. Five different treatments including biocontrol agent and fungicides were made as preventive treatments before the disease appearance and at 25% disease incidence as curative treatments at their recommended doses against apple powdery mildew. Treatments and their doses per litre of water are given in Table 1. Three sprays were carried out at 15 days interval basis. All spray treatments were made with a high-volume applicator with hand machine. A pressure of 2,000 kPa was used and plants were sprayed till run-off, approximately 16 liter of spray mix per plant. Tagged panicles were evaluated for the incidence of powdery mildew (percent), 15 days after the last spray, calculated on the basis of the inflorescences/panicles affected.

Data were statistically analyzed for mean differences using statistical software Statistix 8.1 as described by Steel, *et al* (22).

RESULTS AND DISCUSSION

Symptomatology and pathogen identity

Signs of the disease are dependent upon the susceptibility and resistance of the variety, time and degree of the infection and weather conditions. It was recorded that frequency of the disease was high on leaves and flowers with more than 70% relative humidity commonly present in the microclimate of lower leaf surface at temperature ranged between 10 to 25^{o}C In contrast to most foliar diseases leaf wetting is a deterrent to infection. Same observations has been recorded earlier (9). In case of leaf infection, young leaves were infected more than the older one and lesions on the upper surface of infected leaf appeared as powdery white in colour and then turned into darker brown in colour. Curling/rolling and crinkling of infected leaves

were also observed in few leaves as described in previous study (26). In case of shoot infection, shoots appeared diminutive and young shoots become dead. Same symptoms were also recorded by earlier scientists (10, 24). In case of flowers and fruit infection, small portion of blooming petals, sepals, receptacles and peduncles were found sick with white powdery mass of the fungus. Diseased blooms will fail to set fruit and or produce underdeveloped, rusted fruit. At the time of fruit harvest, few russeted apple fruit were also observed from the plants, in the untreated control treatment. Same observation has been recorded by (4, 10, 11, 12, 26, 28),. From the superficial powdery mass on diseased plant tissues /flowers/panicles/shoot buds, mycelium containing asexual conidia on conidiophores were observed and identified as of *Podosphaera leucotricha* as briefed in the (9, 17).

Comparative efficacy of biocontrol agent and fungicides

All tested treatments significantly reduce the disease incidence as compared to un-treated control treatment. In case of treatments applied as preventive sprays at their recommended doses, Taegro (*Bacillus subtilis*) @ 6 g per litre.water gave 70.27 percent disease control (mean incidence 9.44%) and with enhanced dose (12 g per litre water), it gave 78.07 percent control (mean incidence 7.00%) (Table 2). Mean incidence in untreated control treatment remained as 33.22 percent.. In case of curative treatment, the product (6 g/litre water) at the time of more than 25 percent disease incidence gave disease control upto 37.4 percent (mean incidence 24.89%) while at enhanced dose (12 g/litre water), it gave 44.52 percent disease control (mean incidence 22.22 percent) (Table 2) Mean disease incidence in untreated control treatment remained as 40.00 percent Utkhade and Koch (25)observed in their studies that *Bacillus subtilis* significantly reduced powdery mildew severity in cucumber when compared with untreated plants although other non-chemical products tested provided better results than *B. subtilis.* Another (14) study suggested use of Bacillus based biocontrol agents in specific climate. *Bacillus subtilis* isolates AG704 and HG77 proved good colonizer in apple fields but poor colonizer in storage conditions against apple fruitrot control. In another study conducted (6) Bacilli foliar spray treatments showed a wide potential for apple quality improvement and as effective as plant growth promoting Rhizobacteria widen the range of their availability for orchard application. Marine *et al*.(15) described the effect of biological compounds produced by Bacilllus strains and considered their effectiveness is somewhat inconsistent. In case of fungicide Sulfex gold 80 WP (Sulfur) it use use as preventive application against the disease, gave 72.78 percent disease control with mean incidence of 8.89 percent but as

curative application at 25 percent disease incidence, it gave control upto 39.76 percent with mean disease incidence of 23.89 percent (Table 2).

Table 1. Chemicals and Treatments made on apple against powdery mildew

Treat-ments	Formulation	Active ingredient	Dose/lit. H_2O	Source
T1	Taegro	**Bacillus subtilis*	6.00 gm	Novozymes Biologicals Inc. USA.
T2	Taegro	**Bacillus subtilis*	12.00 gm	Novozymes Biologicals Inc. USA.
T3	Sulfex gold 80% WP	Sulfur	5.00 gm	Jafar Brothers, Pakistan Pvt Ltd
T4	Topass 100% EC	Penconazole	0.50 ml	SyngentaPakistan Pvt Ltd.
T5	Topsin M 72% WP	Thiophanate methyl	2.00 gm	SyngentaPakistan Pvt Ltd
T6	Untreated Control	-	-	-

* Bacillus subtilis var. amyloliquefaciens strain FZB24: 24.5% in a corn starch formulation

Table 2. Comparative efficacy of bio control agent and fungicides against apple powdery mildew (Mean incidence & % Disease reduction).

Treatment	Formulation	Preventive treatments		Curative treatments	
		Mean Disease incidence	% Disease reduction	Mean Disease incidence	% Disease reduction
T1	Taegro	9.44b	70.27	24.89b	37.43
T2	Taegro	7.00b	78.07	22.22b	44.52
T3	Sulfex gold 80%WP	8.89b	72.78	23.89b	39.76
T4	Topass 100% EC	5.67b	82.96	10.00c	75.02
T5	Topsin M 72% WP	7.22b	76.88	12.44c	68.51
T6	Untreated Control	33.22a	0.00	40.00a	0.00
L.S.D. at P 0.05		6.0561		4.0565	

Marine *et al.(15)* too described the use of inorganic sulfur as preventive strategy against apple powdery mildew. According to another study (27) Kumulus D. F. (sulfur) performed well in indoor experiments against apple powdery mildew but use of sulfur in the field is restricted because it is an irritant that can cause asthma-like reactions and allergies (6). Topass 100 EC (Penconazole) when used as preventive spray, gave 82.96 percent control with mean incidence of 5.67 percent. However this systemic fungicide as curative, gave 75.02 percent powdery mildew control as the incidence remained as 10.00 percent. Nasie *et al* (17) described the long protective and curative activity of Penconazole against a broad spectrum of powdery mildews. Topsin M 72 WP (Thiophenate methyl) when used as preventive

spray against the disease, it gave control to 76.88 percent with mean incidence of 7.22 percent However this fungicide when applied as curative, gave 68.51 percent control and mean incidence remained as 12.44 percent. In a perivious study (17). Benomyl, Thiophanate, Carbendazim and Thiabendazole are stated to be effective against a wide range of plants diseases including powdery mildews. Nasir *et al*. (18) described the same effect of Contaf Plus 051 SC (Hexaconazole) against apple powdery mildew. All these have protective and eradicative properties but pose resistance problems in a number of fungal species.

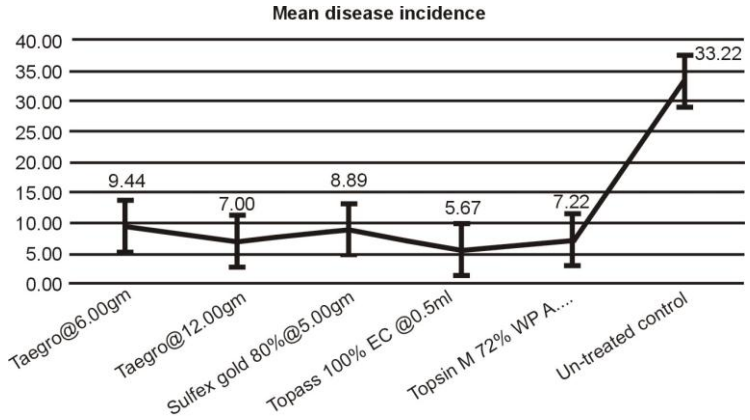

Fig. 1. Mean apple powdery mildew incidence in different treatments (Preventive treatments)

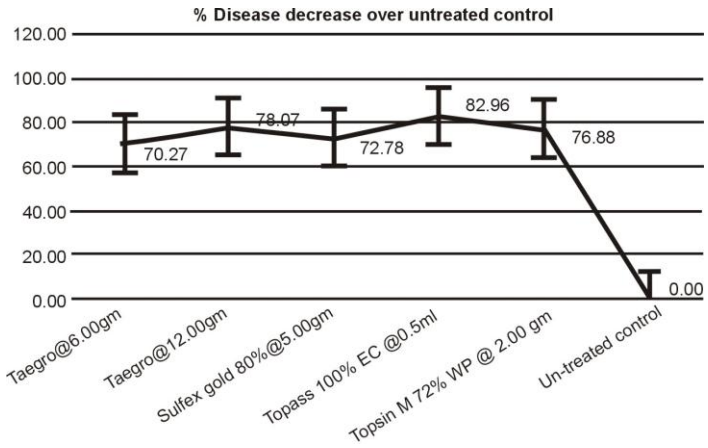

Fig.2: % Disease reduction in different treatments (Preventive treatments).

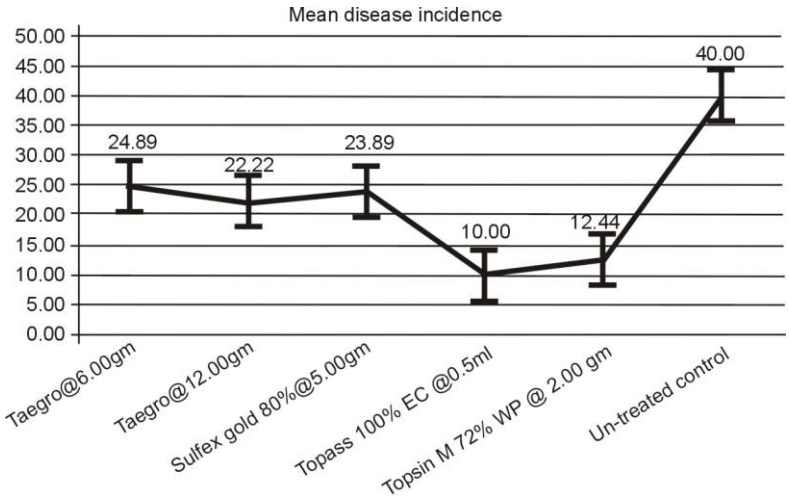

Fig. 3. Mean apple powdery mildew incidence in different treatments (Curative treatments)

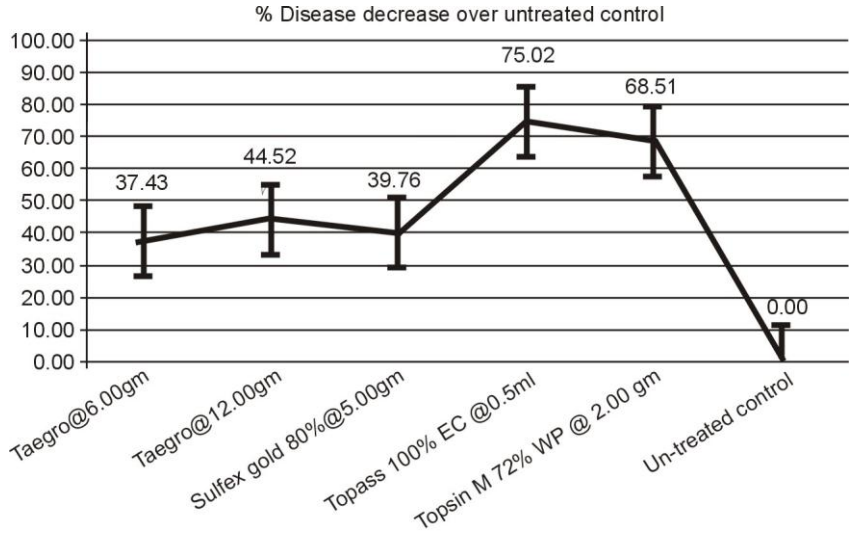

Fig. 4. % Disease reduction in different treatments (Curative treatments)

CONCLUSION

Mycoparasite based product Taegro (*Bacillus subtilis*) may reduce the disease effectively as compared to untreated control treatment, when applied as a disease preventive strategy, similar to other preventive fungicides applied against apple powdery mildew. This product may be utilized in

integrated disease management programme, as an alternate source to toxic fungicides.

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