Integrated disease management of chilli anthracnose

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ABSTRACT

The present studies were undertaken to investigate the effect of bioprotectant, plant based chemical like Salicylic acid and silicon based nutrient Potassium silicate against the anthracnose or ripe fruit rot of chilli (*Capsicum annuum* L.) incited by *Colletotrichum capsici* (Sydow.) Butler and Bisby in chilli var. K2. The chilli anthracnose disease susceptible variety Kovilpatti-2 (K2) grown in pots and field trial were used for the study. The plants were given artificial inoculation by spraying the spore suspensions after pinpricking the fruits with adequate spore load at 90-days after transplanting for fruit rot incidence assessment. The crop was maintained in a poly house with frequent spraying of water to provide adequate moisture and relative humidity to enable successful infection by the pathogen. Three replications for each treatment and a control were maintained. The fungicide mancozeb @ 0.25 per cent was used for comparison and the standard agronomic practices as recommended by the State Agricultural Department were followed. The plant activator and silicon-based nutrient were sprayed as individual as well as combined approach manner. Seed treatment with *Pseudomonas fluorescens* @ 10 g/kg of seeds in all the treatments except comparison fungicide and control treatments. Among the various treatments, seed treatment with Bio protectant, foliar application of Salicylic acid @ 50 ppm on 40 days after transplanting (40 DAT) and foliar application of Potassium silicate @ 3 % on 60 DAT recorded the minimum disease incidence and increased fruit length, fruit weight, fruit per plant, branches per plant, plant height and germination percentage when compared to control and Comparison fungicide Mancozeb. The activity of plant defense enzymes like Peroxidase, polyphenol oxidase and Phenylalanine ammonia lyase increased up to 5th day of sampling in plants treated with Bio-protectant, resistance inducing chemical along with potassium silicate and challenged inoculated with the test pathogen. Also, the same treatment significantly decreased the disease severity and increased the growth and yield parameters when compared to control and comparison fungicide under the field trials.

Key words: Biocontrol agent, Plant activator, Silicon based nutrient, Integrated disease management

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INTRODUCTION

Chilli crop is affected by several fungal, bacterial and viral diseases, of which chilli anthracnose causes considerable damage, inflicting severe quantitative and qualitative losses (Anand *et al*., 2009; Anand *et al*., 2010). The estimated loss due to this disease ranged from 8 to 60 percent in different parts of India (Pandey and Pandey, 2003). The disease is caused by fungus *Colletotrichum capsici* Bisby and Butler that infect both unripe and ripe chilli fruits (Krairuan *et al.*, 2008). *C.capsici* can survive in and on seed as acervuli and microsclerotia (Montri *et al.*, 2009). *C. capsici* infection will be higher in the mature stage of chilli plant than in the early stage of plant (Krairuan *et al.*, 2008). The disease is both seed borne and air borne and affects seed germination and vigour to a greater extent (Saxena *et al.*, 2016).

The possible management practices of anthracnose disease are the use of fungicides and biological agents**.** Use of fungicide to control the disease because several adverse effect *i.e.* development of resistance in the pathogen, residual toxicity, pollution in the environment, high cost (Bajipai and Kang, 2012). Therefore, it has become necessary to adopt ecofriendly approaches for better crop health and yield. In addition to biocontrol agents, induced resistance by chemicals may provide an efficient approach to plant protection. Resistant inducing chemicals are known as inducers of phytoalexins and/or elicitors of resistance in different plant species (Biswas *et al.,* 2008; Shabana *et al.,* 2008; Hadi and Balali, 2010). Several chemicals *viz.,* Salicylic acid (Sarwar *et al*., 2011; Jaiganesh, 2012), Acibenzolar–S–Methyl (Bengtsson *et al.,* 2008), Acetyl Salicylic acid (White, 1979), Nicotinic acid (Jaiganesh, 2005), Jasmonic acid (Cohen *et al.,* 1993) and Oxalic acid (Toal and Jones, 1999) have shown induced resistance in various crops.

Besides, a promising alternative for the control for many plant diseases, including Anthracnose, is the application of silicon (Si) to soils deficient in this element (Datnoff *et al*., 2007; Rodrigues and Datnoff, 2015). In recent years, silicon (Si) is being used for the control of fungal diseases with promising results (Yanar *et al.,* 2011) and silicon accumulation has been reported to be one of the main factors responsible for enhanced resistance against various pathogens of crop plants (Junior *et al*., 2009).

Single use of fungicidal spray or management practice will not be effectively used for the management of chilli anthracnose. Therefore, with an aim to develop an integrated strategy involving the use of bio protectant, silicon based nutrient and resistance inducing chemical for the successful sustainable management of chilli anthracnose.

Materials and Methods

Collection and isolation of the pathogen

Fruits of chilli variety K2 (Kovilpatti variety) affected with fruit rot was collected from different places of Tamil Nadu *viz.,* Kovilpatti, Vallampadugai, Sivapuri, Uthiramerur, Chengalpattu, Ariyalur, Villupuram, Musiri, Tindivanam, Cuddalore, Thiruvannamalai and Theni were used for isolation. The isolation of the pathogen was done by following standard tissue isolation method (Than *et al.,* 2008).

Identification of the pathogen

The morphological character of *C.capsici* such as acervulus, setae and conidia were studied. These characters were compared with the culture of *C.capsici* obtained from ITCC, IARI, New Delhi.

Survey for occurrence of fruit rots causes by *Colletotrichum capsici*

A field survey was conducted to assess the extent of fruit rot occurrence of chilli in major chilli growing areas of Tamil Nadu state during 2018. The places *viz.,* Kovilpatti, Vallampadugai, Sivapuri, Uthiramerur, Chengalpattu, Ariyalur, Villupuram, Musiri, Tindivanam, Cuddalore, Thiruvannamalai and Theni where chilli is traditionally grown are selected for assessing the prevalence of fruit rot disease caused by *C. capsici*. During survey plants affected due to fruit rot disease was found and also the total number of plants observed were counted and recorded. The percent disease incidence was worked out as per Phytopathometry (Mayee and Datar, 1986). Also the infected plants showing the typical symptoms of leaf spot and fruit rot due to infection with *C. capsici* were collected for isolation of the pathogen.

Isolation of *C. capsici*

The diseased chilli fruits showing the typical symptoms of fruit rot disease were collected fresh from 10 conventional chilli growing areas of Tamil nadu. The pathogens were isolated on potato dextrose agar (PDA) medium from the diseased specimen showing the typical symptoms. The infected portion of the fruit was cut into small bits, surface sterilized in 0.1 per cent mercuric chloride solution for 30 sec. washed in repeated changes of sterile distilled water and plated onto PDA medium in Petri dishes. The plates were incubated at room temperature (28 ± 2^0c) and were observed the fungal growth. The fungus was purified by single spore isolation technique (Rangaswami, 1972) and

identification of the isolates was confirmed by comparing with the culture (ITCC No. 6071) obtained from ITCC, IARI, New Delhi and the purified isolates were maintained on PDA slants for further studies. The pathogen isolated from each of these localities formed an isolate of *C. capsici*.

Virulence in pot culture condition

Five kilograms of top soil collected from chilli growing field was steam pasteurized and filled in 30 cm diameter earthen pots. One month old seedlings of var. K2 were transplanted in pots. The spore suspension (5×10^{-5} mL^{-1}) of *C.capsici* was prepared from 20 days old culture grown on PDA slant using sterile distilled water (Rajapakse, 1998). Ninety days old plants were inoculated with *C. capsici* by spraying spore suspension after pinpricking method. The inoculated plants were incubated in green house for 10 days to observe lesion development on the surface and the intensity of fruit rot was calculated as per cent disease index (PDI) as per the grade chart proposed by Ravinder Reddy (1982) using the formula proposed by Mc Kinney (1923). The per cent disease index (PDI) was calculated using Mc Kinney (1923) infection index.

Pot culture studies

Pot culture studies were conducted to test the efficacy of certain resistance inducing chemicals and bio control agent for assessing their influence on the incidence of chilli anthracnose disease. The chilli anthracnose disease susceptible variety Kovilpatti-2 (K2) grown in pots was used for the study. The plants were given artificial inoculation by spraying the spore suspensions after pinpricking the plants with adequate spore load (5×10^{-5} conidia/mL) at 90 DAT in the evening hours. The crop was maintained in a poly house with frequent spraying of water to provide adequate moisture and relative humidity to enable successful infection by the pathogen. The components were sprayed individually at disease initiation stage and repeated once at fifteen days interval. Three replications for each treatment and a control were maintained. The fungicide mancozeb @ 0.25 per cent was used for comparison and the standard agronomic practices as recommended by the State Agricultural Department were followed.

The effective treatments observed in different experiments (screened trials) conducted under pot and field conditions were pooled together and a new schedule of treatments in combination was evolved for the effective management of fruit rot of chilli. Also, Seed treatment with *Pseudomonas fluorescens* @ 10 g/kg of seeds was applied to the entire treatments (PfS) except control and comparison fungicide. The treatment details are: T_1 – PfS + PfF_1 + PfF_2, T_2 – PfS+ SA_1 + SA_2, T_3 – PfS+ PS_1 + PS_2, T_4 – PfS + PS_1 + SA_2, T_5 – PfS + SA_1 + PF_2, T_6 – PfS + SA_1 + PS_2, T_7 – PfS + PS_1 + PF_2, T_8 – PfS + PfF_1 + SA_2, T_9 – PfS + PfF_1 + PS_2, T_{10} – Mancozeb 50 WP @ 0.25 per cent as foliar spray (comparison) and T_{11} – Control.

Where, PfF_1, SA_1, PS_1 –Foliar application on 40 DAT; SA–Salicylic acid; PS–Potassium silicate; PfF_2, SA_2, PS_2 - foliar application on 60 DAT; SA - Foliar spray of Salicylic acid @ 50 ppm; PS– Foliar spray of Potassium silicate @ 3 %; PfF–Foliar spray of *P.fluorescens* @ 0.5 %)

Disease incidence

The fruit rot incidence was assessed on 100^{th}, 125^{th}, and 150^{th} day after transplanting and the leaf spot incidence was calculated on 80^{th} day. The intensity of fruit rot was calculated as per cent disease index (PDI) following the grade chart proposed by Ravinder Reddy (1982) using the formula by McKinney (1923) were described as earlier.

Enzyme extraction

One g of the leaf material cut into small bits was crushed in chilled 0.1 M sodium phosphate buffer at pH 7.1. The volume was made up to 5 ml with the buffer, centrifuged at 2,100 rpm. for 30 min. and the supernatant was used as the enzyme source and all the assays *viz.*, polyphenol oxidase, peroxidase and phenylalanine ammonia lyase were performed in a UV Spectrophotometer at 28±2°C (Sridhar *et al.,* 1969). Polyphenol oxidase (PPO) (Mayer *et al.,* 1965), Peroxidase (PO) (Hammerschmidt *et al.,*

Table 1. Survey of disease incidence of chilli Anthracnose in different locality of Tamil Nadu during 2018

Locality	Crop stage	Variety	Disease incidence (%)	Pot culture condition Per cent disease incidence (Pathogenicity)
Cc1- Kovilpatti	Fruit stage	K2	19.2	82.88
Cc2- Vallampadugai	Flowering	K1	5.6	54.82
Cc3- Sivapuri	Fruit stage	CO-1	4.5	63.50
Cc4- Uthiramerur	Fruit stage	K2	15	80.76
Cc5- Chengalpattu	Flowering	K2	16.4	83.50
Cc6- Ariyalur	Flowering	MDU-1	7.2	38.54
Cc7- Viluppuram	Flowering	Jwala	8.9	45.06
Cc-8 Musiri	Fruit	CO-1	8.5	49.75
Cc-9 Tindivanam	Fruit	K2	12.8	81.60
Cc-10 Theni	Flowering	K1	8.6	56.80
Cc – 11 Cuddalore	Flowering	MDU-1	8.2	43.58
Cc-12 Thiruvannamalai	Flowering	Palur	7.5	27.06

1982) and Phenylalanine ammonia lyase (PAL) (Ross and Sederoff, 1992) were estimated. Enzyme activity of PO and PPO was expressed in terms changes in absorbance /minute/mg of protein (Anonymous, 1965). The activity of PAL was expressed as nmol transcinnamic acid min^{-1} mg $protein^{-1}$.

Results and Discussion

The results of the survey revealed that among the ten major regions, the maximum and minimum mean per cent disease incidence was recorded in Kovilpatti region and Sivapuri respectively. The variation in the extent of the disease incidence might be due to the prevalence of the isolates of the pathogen differing in their virulence and the susceptibility of the host. Also, the results of the present study are similar to the findings of Pandey (2003) who reported maximum disease incidence in Punjab and attributed growing of susceptible variety as the reason. In the present survey, the disease incidence was high in fruit ripening stage of the crop. Similar such observation was made by Sujatha Bai (1992) who reported that secondary infection of chilli fruit mostly occur in matured ripening fruit.

The results presented in table 1 indicated that each isolate could infect chilli fruits and produce typical symptoms of fruit rot, indicating their pathogenic potential on chilli. Pathogenicity tests revealed that these isolates expressed different levels of virulence and of the 10 isolates of *C. capsici* the isolates Cc5, Cc1 and Cc9 were highly virulent which recorded 83.50 %, 82.88 % and 81.60 % of fruit rot incidence respectively. *C. capsici* isolate Cc12 was the least virulent. This finding was in conformity with the findings of Ali *et al.* (2002) who reported that virulence of pathogen differed from locality to locality with the change of temperature, humidity and rainfall.

Results (Table 3) of the study showed that, the fruit rot disease incidence was effectively controlled by the combined application Seed Treatment (ST) with *P. fluorescens* @ 10g/Kg of seeds and Foliar application of Salicylic acid @ 50 ppm on 40 DAT and foliar application of Potassium silicate @ 3 % on 60 DAT which showed 14.38, 10.08, 06.74 on per cent fruit rot incidence on 100^{th}, 125^{th} and 150^{th} day of observation respectively. The leaf spot incidence also was minimum in the treatment T_6. It was followed by seed treatment with *P.fluorescens* and foliar spray of salicylic acid @ 50 ppm on 40 DAT along with foliar spray of *P.fluorescenes* @ 0.5 % on 60 DAT as compared to control.

The results of Pot and field trial experiments were given in table 2 and 5. All the treatments controlled the disease significantly but with varying degrees of intensity. Significant reduction in the disease incidence was observed in the combined application of Bio

Table 2. Effect of Pf, SA and PS on Biometrics characters of Chilli var. K-2 under pot culture conditions **130**

Treatments	Mean shoot length (cm)	Mean no. of flowers/ plant	Mean Branches per plant	Mean no. of fruits/plant	Mean fruit length (cm)	Fruit yield (t/ha)	
						Fresh (wt.)	Dry (wt.)
PfS + PfF_1 + PfF_2	76.91	58.30	21.80	40.28	11.06	4.09	2.49
PfS+ SA_1 + SA_2	74.66	70.68	23.00	46.73	12.05	4.54	3.19
PfS+ PS_1 + PS_2	67.16	55.73	20.92	37.56	10.85	3.91	2.12
PfS + PS_1 + SA_2	65.51	51.42	20.36	34.24	10.23	3.76	2.01
PfS + SA_1 + PF_2	80.87	75.68	23.56	50.62	12.28	4.97	3.71
PfS + SA_1 + PS_2	81.54	77.39	25.72	51.45	12.60	5.06	3.83
PfS + PS_1 + PF_2	72.06	62.76	22.17	41.76	11.38	4.21	2.83
PfS + PfF_1 + SA_2	78.94	72.16	23.16	48.80	12.14	4.81	3.58
PfS + PfF_1 + PS_2	74.18	68.52	22.68	43.82	11.76	4.38	3.01
Mancozeb	62.32	45.67	20.46	35.60	8.90	4.17	3.14
Control	46.86	25.62	13.45	12.30	6.54	1.89	1.36
S.E.	0.037	0.024	0.039	0.027	0.018	0.024	0.019
CD (P=0.5)	0.07	0.05	0.07	0.05	0.08	0.06	0.05

control agent, resistance inducing chemical and Silicon based nutrient. Among the different treatments tested at T_6 (ST with *P.fluorescens* @ 10 g/kg of seeds and foliar spray of Salicylic acid @ 50 ppm on 40 DAT along with Foliar spray of Potassium silicate @ 3 % on 60 DAT) was the most effective treatment in controlling the disease incidence when compared to control and comparison fungicide. The same treatment recorded the Maximum shoot length, number of flowers per plant, Number of branches per plant, Number of fruits per plant, maximum fruit length and fruit yield of both fresh and dry weight.

The resistance inducing chemicals, bio control agent and silicon based nutrient were sprayed at different dosages in chilli plants and changes in the peroxidase enzyme activity was observed (Table 4). It was inferred that application of Seed treatment with *P. fluorescens* @ 10 g/kg of seeds, foliar application of Salicylic acid @ 50 ppm on 40 DAT along with foliar spray of potassium silicate @ 3 % on 60 DAT led to an increase in PO activity up to 5th day after challenge inoculation when compared to control and comparison fungicide. The PO activity reached maximum levels on 5th day after challenge inoculation of the pathogen. The increased activity of PPO was observed in plants challenge inoculation with anthracnose in chilli. Application on Bio inoculant, Salicylic acid along with Potassium silicate led to increased PPO activity up to 5th day when compared to control (Table 4).

Table 3. Effect of Pf, SA and PS on Leaf spot and fruit rot incidence under pot culture conditions

Treatments	Leaf spot incidence	Fruit rot incidence on 100th day	Fruit rot incidence on 125th day	Fruit rot incidence on 150th day
PfS + PfF_1 + PfF_2	10.06	16.80	14.02	11.34
PfS+ SA_1 + SA_2	06.78	15.40	12.47	09.17
PfS+ PS_1 + PS_2	10.18	17.96	14.96	12.08
PfS + PS_1 + SA_2	10.92	20.06	16.37	14.36
PfS + SA_1 + PF_2	05.93-	14.72	10.94	08.10
PfS + SA_1 + PS_2	05.64	14.38	10.08	06.74
PfS + PS_1 + PF_2	08.92	16.41	13.30	10.95
PfS + PfF_1 + SA_2	06.21	14.96	11.46	08.68
PfS + PfF_1 + PS_2	07.53	15.84	12.90	10.32
Mancozeb	18.02	26.03	21.16	16.90
Control	41.86	53.42	65.06	72.49
S.E.	1.22	1.08	1.52	1.89
CD (P=0.5)	2.23	2.19	2.67	2.92

It was followed by Seed treatment and foliar spray Bioinoculant along with Plant activator Salicylic acid treated plants challenge inoculated with *C. capsici*. Plants inoculated with pathogens alone recorded comparatively less PPO activity and the activity was very low on 5th day after inoculation, also PPO activity decreased with age in control. The activity increased with age more in plants applied with Bio control agent, Salicylic acid and Potassium silicate, thus establishing it's antagonistic and controlling effect on fruit rot disease. The results revealed that ST with *P.fluorescens* and foliar spray of Salicylic acid @ 50 ppm on 40 DAT along with foliar spray of Potassium silicate @ 3 % @ 60 DAT activated PAL in leaf of the chilli plants. *C. capsici* inoculation was found to induce a apid and transient accumulation of PAL at the site of infestation.

Table 4. Changes in Plant defense activity in *C. capsici* challenged inoculation in chilli plants treated with different components under pot culture condition

Treatments	PO activity in plants				PPO activity in plants				PAL activity in plants			
	Time Interval (days)				Time Interval (days)				Time Interval (days)			
	0	1	5	10	0	1	5	10	0	1	5	10
PfS + PfF_1 + PfF_2	0.26	0.42	0.76	0.52	0.25	0.63	0.91	0.53	0.51	0.70	0.91	0.51
PfS+ SA_1 + SA_2	0.36	0.55	0.12	0.76	0.31	0.81	1.13	0.81	0.65	0.75	1.07	0.79
PfS+ PS_1 + PS_2	0.23	0.39	0.69	0.49	0.24	0.54	0.73	0.51	0.47	0.67	0.79	0.47
PfS + PS_1 + SA_2	0.21	0.36	0.56	0.46	0.23	0.51	0.59	0.39	0.41	0.63	0.61	0.45
PfS + SA_1 + PF_2	0.41	0.63	1.30	1.00	0.41	0.93	1.28	1.00	0.69	0.81	1.14	0.98
PfS + SA_1 + PS_2	0.43	0.69	1.35	1.09	0.43	0.97	1.35	1.13	0.71	0.85	1.20	1.03
PfS + PS_1 + PF_2	0.29	0.47	0.90	0.59	0.26	0.71	1.01	0.61	0.56	0.71	0.98	0.59
PfS + PfF_1 + SA_2	0.38	0.56	1.19	0.89	0.37	0.86	1.21	0.89	0.67	0.79	1.11	0.91
PfS + PfF_1 + PS_2	0.33	0.51	1.01	0.63	0.29	0.75	1.06	0.67	0.61	0.78	1.03	0.71
Mancozeb	0.34	0.54	0.96	0.79	0.26	0.49	0.86	0.85	0.56	0.69	0.93	0.81
Control	0.12	0.16	0.20	0.17	0.19	0.26	0.17	0.13	0.33	0.31	0.43	0.39

Moreover, PAL activity increased with increase in time *i.e.,* 5th day after pathogen infection. Plants inoculated with pathogen alone showed increased activity of PAL for 3-5 days and thereafter the activity declined drastically (Table 4).

PPO is a copper containing enzyme, oxidizing phenolics to highly toxic quinines and involved in the terminal oxidation of diseased plant tissues which was attributed for its role in disease resistance (Kosuge, 1969). Higher PPO activity was related to higher contents of phenolic compounds, which have been shown to provide resistance against diseases (Sharma *et al.,* 1994). The Peroxidase and Polyphenol oxidase are reported to have important roles in plant disease resistance (Zhang *et al.*, 2007).

Phenylalanine Ammonia Lyase (PAL) is the first enzyme of the phenylpropanoid pathway and is involved in the biosynthesis of phenolics, phytoalexins and lignins (Qin and Tian, 2005). Vimala and Suriachandraselvan (2009) reported earlier and increased activities of PAL in salicylic acid pre-treated bhendi plants challenge inoculated with *Erysiphe cichoracearum.* Exogenous application of silicon treated rice plants inoculated with *Pyricularia oryzae* significantly increased the activities of Peroxidase, Polyphenol oxidase and Phenylalanine ammonia lyase (Cai *et al.,* 2008).

Table 5. Effect of Pf, SA and PK on Fruit rot of chilli and growth, yield attributes under field condition

Treatments	Mean shoot length (cm)	Mean no. of flowers/plant	Mean no. of fruits/plant	Mean fruit length (cm)	Fruit yield (t/ha)		Fruit rot incidence on 150th day
					Fresh(wt.)	Dry(wt.)	
PfS + PfF_1 + PfF_2	70.16	63.45	42.06	9.13	3.81	2.43	15.76
PfS+ SA_1 + SA_2	76.91	70.19	51.04	10.39	4.57	3.10	5.89
PfS+ PS_1 + PS_2	68.91	60.53	40.51	8.74	3.59	2.11	19.34
PfS + PS_1 + SA_2	66.14	57.37	37.93	8.09	3.32	1.90	21.58
PfS + SA_1 + PF_2	80.41	75.69	57.16	11.12	5.14	3.56	3.98
PfS + SA_1 + PS_2	83.50	77.06	59.60	11.36	5.38	3.78	3.63
PfS + PS_1 + PF_2	72.80	66.09	45.21	9.60	4.04	2.69	12.64
PfS + PfF_1 + SA_2	78.76	72.81	54.80	10.73	4.84	3.32	4.30
PfS + PfF_1 + PS_2	74.16	68.35	47.63	10.15	4.29	2.93	8.13
Mancozeb	60.06	65.06	42.58	16.48	3.48	2.46	14.42
Control	36.52	23.47	17.54	7.10	1.61	1.04	48.60
S.E.	0.037	0.81	0.43	0.63	0.52	0.68	1.36
CD (P=0.5)	0.05	0.53	0.08	0.21	0.43	0.25	2.43

Menzies *et al.* (1992) mentioned the formation of a coating on the leaves after spraying potassium silicate, suggesting that this "film" would strengthen the cuticle activity as a mechanical barrier to pathogen penetration. It is logical that reduced disease severities would be at least partially responsible for increased yield. However, silicon in the absence of disease may also increase yield solely as a plant nutrient. Increased yield is probably a function of both reduced disease and more favourable plant nutrition (Datnoff *et al.,* 2001). Thus, foliar application of silicon is a practice that perfectly fits in with eco-friendly strategies for sustainable management of crop diseases (Rezende *et al*., 2009). These earlier reports are in accordance with the present findings.

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