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Original Article

Distribution, host range and toxicity assessment of different insecticides on *Bactrocera diversa* Coquillett, 1904 (Diptera: Tephritidae)

Avaliação da distribuição, gama de hospedeiros e toxicidade de diferentes inseticidas em *Bactrocera diversa* Coquillett, 1904 (Diptera: Tephritidae)

N. Nazir[a*], M. Imran[a], I. Bodlah[b], K. Mahmood[a], M. R. Khan[a], K. Osman[c], A. Rasool[d], M. Usman[a] and A. U. Din[a]

[a]University of Poonch Rawalakot, Faculty of Agriculture, Department of Entomology, Rawalakot, Azad Jammu & Kashmir, Pakistan
[b]Pir Mehr Ali Shah Arid Agriculture University, Department of Entomology, Rawalpindi, Pakistan
[c]Alexandria University, Faculty of Agriculture, Department of Pesticide Chemistry & Toxicology, Alexandria, Egypt
[d]University of Swat, Centre for Biotechnology and Microbiology, Charbagh, Pakistan

Abstract

The present study was conducted to investigate the array of hosts, distribution and to evaluate the toxicity of four insecticides: imidacloprid, fipronil, cypermethrin and chlorpyrifos alone and in combination against 3rd instar maggot and adult stage of fruit fly *Bactrocera diversa* Coquillett, 1904 (Diptera: Tephritidae) during 2021. *B. diversa* maggots were found vigorously feeding inside the cucurbit hosts (pumpkin, cucumber, bitter gourd, watermelon, round melon, bottle gourd) collected from different localities of Poonch division of Azad Jammu & Kashmir, Pakistan, and this species is reported for the first time as new record to this region. Susceptibilities of *B. diversa* to insecticides were evaluated using topical method. Mortality was checked after 3, 6, 8 and 24h of exposure. Cypermethrin was most effective to kill 50% of both larval and adult stage with least LC_{50} [7.2(1.040±0.214), 17.4(0.748±0.193)], respectively followed by imidacloprid. Imidacloprid most effectively killed 90% of both larval and adult population with least LC_{90} value [73.2 (3.013±0.708) 16.9 (1.886±0.437)] respectively after 24 hours. Cypermethrin with chlorpyrifos most effectively killed 50 and 90 percent of both larval and adult stage of *B. diversa* with least LC_{50} value [11.3 (1.085±0.245), 2.5 (0.759±0.252)] and least LC_{90} value [171.3 (1.085±0.245), 121.9 (0.759±0.252)], respectively after 24h of exposure. Toxicity of each insecticide increased with exposure for longer time and increased dose. Cypermethrin is suggested as most effective against both larval and adult stages of *B. diversa* in combination with chlorpyrifos followed by imidacloprid.

Keywords: *Bactrocera diversa*, cypermethrin, fruit fly, insecticides, toxicity.

Resumo

O presente estudo foi conduzido para investigar a variedade de hospedeiros, distribuição e avaliar a toxicidade de quatro inseticidas: imidaclopride, fipronil, cipermetrina e clorpirifós isoladamente e em combinação contra larva de 3º ínstar e estágio adulto da mosca-das-frutas *Bactrocera diversa* Coquillett, 1904 (Diptera: Tephritidae) durante 2021. Larvas de *B. diversa* foram encontradas se alimentando vigorosamente dentro dos hospedeiros de cucurbitáceas (abóbora, pepino, cabaça amarga, melancia, melão redondo, cabaça) coletados em diferentes localidades da divisão Poonch de Azad Jammu e Caxemira, Paquistão, e essa espécie é relatada pela primeira vez como novo registro para essa região. A suscetibilidade de *B. diversa* a inseticidas foi avaliada por método tópico. A mortalidade foi verificada após 3, 6, 8 e 24 horas de exposição. A cipermetrina foi mais eficaz para matar 50% do estágio larval e adulto com menos CL50 [7,2(1,040 ± 0,214), 17,4 (0,748±0,193)], respectivamente, seguido por imidaclopride. O imidaclopride matou mais efetivamente 90% da população larval e adulta com o menor valor de LC90 [73,2 (3,013 ± 0,708) 16,9 (1,886 ± 0,437)], respectivamente, após 24 horas. Cipermetrina com clorpirifós matou mais efetivamente 50% e 90% do estágio larval e adulto de *B. diversa* com valor mínimo de LC50 [11,3 (1,085 ± 0,245), 2,5 (0,759 ± 0,252)] e valor mínimo de LC90 [171,3 (1,085 ± 0,245), 121,9 (0,759 ± 0,252)], respectivamente, após 24 horas de exposição. A toxicidade de cada inseticida aumentou com a exposição por mais tempo e com o aumento da dose. A cipermetrina é sugerida como mais eficaz contra os estágios larval e adulto de *B. diversa* em combinação com clorpirifós seguido de imidaclopride.

Palavras-chave: *Bactrocera diversa*, cipermetrina, mosca-da-fruta, inseticidas, toxicidade.

*e-mail: nailanazir@upr.edu.pk
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1. Introduction

Among most of the economically important insect pests, tephritid fruit flies (Diptera: Tephritidae) are major group of horticultural pests that directly impact on the trade of a country along with losses at national level (Joomaye and Price, 2000; Hashem et al., 2001; Ormbsy, 2021). Approximately 500 known species of genus *Bactrocera* (Diptera: Tephritidae) are endemic to South Pacific Islands and Southeast Asia (Drew and Hancock, 2000). About four thousand species of Tephritidae are known throughout the world, out of which 350 are of economically important (Plant Health Australia, 2016). Seventy *Bacterocera* polyphagous species feeds on commercial vegetables and fruits and pose serious threat to international trade (Garcia, 2009; Clarke et al., 2011; Qin et al., 2015; Vargas et al., 2015; Doorenweerd et al., 2018). These insect pests cause severe damages to fruits and vegetables in Subtropical, tropical and temperate regions of the world (Joomaye and Price, 2000; Hashem et al., 2001) and their infestation recorded in Pakistan is up to 89% (Grewal and Kapoor, 1987), whereas the infestation ranged up to 20% in North Western Himalayan region (Gupta and Bhalla, 1990). Hussain et al. (2010) reported annual loss of approximately 200 million dollars in Pakistan due to Tephritid fruit flies. Another report also illustrated that owing to fruit fly attack Pakistan facing losses of $200 million yearly in form reduction in export of fruits and vegetables (The Express Tribune, 2017). Stonehouse et al. (2002) reported that if loss estimates for all of Pakistan are extrapolated from infestation caused by fruit fly and reductions in fruit and vegetable yield, a gross annual saving of 4915 million Pakistani rupees, or US$144.6 million can be possible.

Fruit fly maggots inside the commodity make it unfit for human consumption especially mangoes (Stonehouse et al., 2002). National and international markets have zero tolerance for such nuisance of pests and reject the commodity which adversely affects the trade of a country and economy (Reynolds et al., 2017). To overcome this problem in Pakistan, insecticides are applied to manage them by various ways such as cover spray in case of heavy infestation, attractant and baiting (FAO, 1986) and many researchers inferred that the most effective pest management strategy is chemical control (Ullah et al., 2012) and majority of growers today use insecticides to combat fruit flies, which has shown some beneficial outcomes (Khan et al., 2022). Application of various insecticides such as organophosphates and pyrethroids decrease their damage but their efficacy reduces with the passage of time (Zhang et al., 2008; Jin et al., 2011). Therefore, factors affecting the sensitivity of insecticides are necessary to know for better management of this economic important pest. Environmental factors such as temperature has a positive correlation with insecticides in insects especially organophosphates (Gao and Zheng, 1989) while pyrethroids have negative correlation with temperature (Grafius, 1986). Host plants also affect the toxicity of insecticides by detoxification of secondary metabolites in insects (Sheets, 2000), insecticide concentration and population density are also notable factors (Musser and Shelton, 2005). In Pakistan, bitter gourd, musk melon, apple, ber, guava, mango, citrus, peach and apricot are heavily infested by fruit flies and toxicity of various insecticides such as emamectin benzoate, trichlorfon, λ-cyhalothrin and imidacloprid have been checked on *B. zonata* (Khan and Naveed, 2017). Hussain et al. (2019) compared the toxicity of Imidacloprid, icetamiprid, flufenoxuron and nitenpyram against *B. zonata.* Efficacy of trichlorfon, lambda-cyhalothrin, and imidacloprid was assessed on *B. zonata* under laboratory conditions (Haider et al., 2021). Various insecticides against have been applied to *Bactrocera zonata, B. dorsalis, B. invedens, B. tyroni, B. minax, B. cucurbitae, Ceratitis capitate* and *Anastrepha fraterculus* for management purpose and toxicity assessments in field and laboratory conditions (Gazit and Akiva, 2017; Nadeem et al., 2012; Lin et al., 2013; Wang et al., 2013; Liu et al., 2015; Rana et al., 2015; Reynolds et al., 2017; Khan and Naveed, 2017; Halawa et al., 2019; Abdullahi et al., 2020; El-Gendy et al., 2021). To control the *Bactrocera* pest species various control and eradication strategies have been opted in different parts of the world which include insecticide application to soil and foliage, release of sterilized males, male annihilation bait sprays and other integrated pest management techniques (Vargas et al., 2015; Scolari et al., 2021). *Zeugodacus diversus* (Coquillett) (syn. *B. diversa* (Coquillett)) is distributed in Pakistan, Sri Lanka, Bangladesh, India, Vietnam, Nepal, Thailand and China (Drew and Romig, 2013; Leblanc et al., 2014; Vargas et al., 2015; Laskar et al., 2016) reported as pest of Cucurbits with losses by oviposition in flowers (Molla et al., 2000; Vargas et al., 2015), decrease yield by dropping infested flowers. It causes serious damage to pumpkin, bottle gourd, ash gourd, ivy gourd, sweet gourd, ribbed gourd, sponge gourd, cucumber, watermelon, snake gourd and bitter gourd (Syed, 1970; Drew and Romig, 2013). In Indo-Pak region including Bangladesh with rise in temperature and flowering of cucurbits this pest become active and causes losses (Kabir et al., 1991; Molla et al., 2000) reported that 42% losses of cucurbits in Bangladesh who is major exporter of bottle gourd, ash gourd and ribbed gourd to Middle East (Naqvi, 2005). however, no literature supporting the insecticidal toxicity assessment on *B. diversa* in field or laboratory conditions.

Poonch division of Azad Jammu & Kashmir is mainly hilly with temperate type climatic conditions where vegetables and fruits supplied mostly from Punjab and Khyber Pakhtunkhwa (KPK), the carrier of fruit fly species in this region. *B. divsera* emerged abundantly from local cucurbit farms (Figures 1-2, Table 1). In the present study, we applied various insecticides to field population of *B. divsera* to check the toxicity along with the investigation of distribution and host range of this pest.

2. Material and Methods

Different infested fruits and vegetables (cucumber, pumpkin, tomato, mango, peach, melon, bitter gourd and summer squash) were collected from untreated local farmer's fields and market places of Poonch Division of Azad Kashmir (Table 1, Figure 2) during June to September of 2021. Collected fruits and vegetables were transported

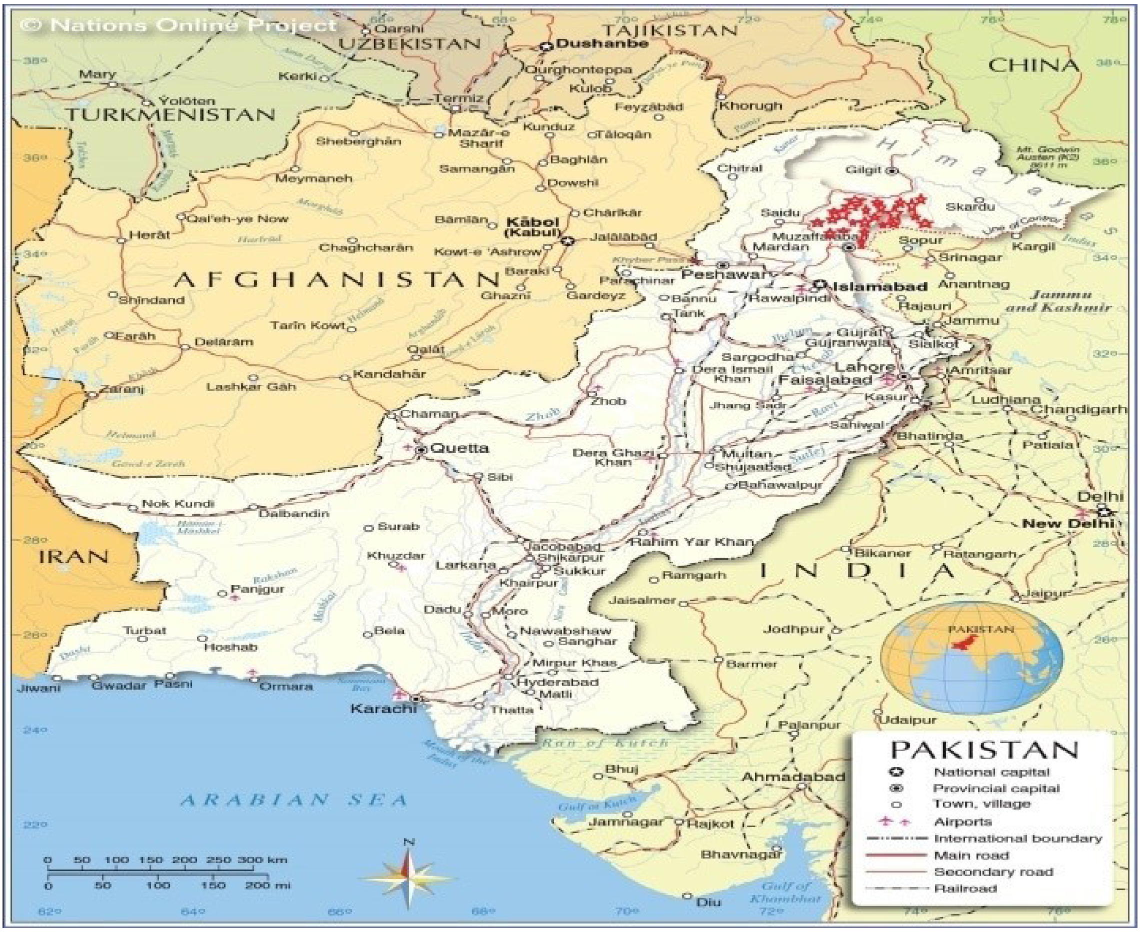

Figure 1. Map of Pakistan showing study area.

to Laboratory of Entomology, The University of Poonch, Rawalakot for rearing and taxonomic identification of fruit fly species. Initially each damage sample was kept separately in plastic container with 1-2 inches moist sand in the bottom. All the containers were labeled with necessary field information, like sample name, location, collection/ rearing date, and collector name. Before separating the damage samples, all the containers were clean with 75% ethyl alcohol to minimize the risk of fungus attack and covered with muslin cloth. These containers were kept at 25+2^{0}C, 65%RH and 16 hours photophase for 2-3 weeks in the laboratory until all fruit flies emerged. After 10-12 days, host samples were checked to make sure that all maggots had left the hosts and goes into sand for pupation. Total numbers of adult fruit flies emerged from pupae were recorded and species were identified on the basis of diagnostic morphological features.

The collected specimens were identified under microscope (Leica MZ6) up to species level with the help of available literature (Mahmood and Hassan, 2005). Pictorial keys of Prabhakar et al. (2012) were used for the process of identification.

2.1. Host range

After identification, most abundantly found species, *Bactrocera diversa* was reared on natural hosts (cucumber, pumpkin, bitter gourd, water melon, bottle gourd, round melon). According to samples collected from different localities of Poonch division of Azad Jammu & Kashmir (Table 1, Figures 1-2) distribution of tephritid fruit flies was also determined.

All the emerged first instar larvae from their natural host were shifted to artificial diet consisting of corn flour, wheat germ flour, yeast powder, agar, sugar, sorbic acid, vitamin C, and linoleic acid (Wang et al., 2013). When all the same age larvae reached at 3rd instar stage, insecticides bioassays were performed. Adult fruit flies were reared on artificial diet consisting of yeast powder, honey, sugar, vitamin C, and water in the rearing cages. Insecticides bioassays on adult fruit flies were performed 2-3 days after pupation.

2.2. Chemicals

Four technical grade insecticides including Chlorpyrifos EC40% (Chlorpyrifos), Arrivo EC10% (Cypermethrin), imidacloprid SC25% (imidacloprid and Agenda EC 25%

Table 1. Selected localities for the sampling of host of *Bactrocera diversa* along with percent losses.

Sr. No	Localities	Longitude Latitude	Altitude (ft)	Hosts	No of Sample	No. of Infested Samples	Losses (%)
1	Rawalakot (Kharick)	33.8584° N 73.7654° E	5430	Pumpkin (*Cucurbita moschata*)	10	4	40
				Cucumber *(Cucumis sativus)*	15	7	46.6
				Bitter gourd (*Momordica charantia*)	9	4	44.4
				Bottle gourd (*Lagenaria siceraria)*	6	2	33.3
2	HaJira (Mandol)	33.7670° N 73.8948° E	2522	Pumpkin (*Cucurbita moschata*)	12	5	41.6
				Cucumber *(Cucumis sativus)*	20	9	45
				Bitter gourd (*Momordica charantia*)	16	7	43.75
				Bottle gourd (*Lagenaria siceraria)*	8	3	37.5
				Round melon (*Praecitrullus fistulosus*)	15	6	40
3	Bagh	33.9794° N, 73.7772° E	3405	Pumpkin (*Cucurbita moschata*)	12	6	50
				Cucumber (*Cucumis sativus*)	18	7	38.8
				Bitter gourd (*Momordica charantia)*	20	9	45
				Watermelon (*Citrullus lanatus*)	5	2	40
				Round melon (*Praecitrullus fistulosus*)	14	6	42.8
				Bottle gourd (*Lagenaria siceraria)*	7	2	28.6
4	Dherkot	34.0390° N, 73.5771° E	5498	Pumpkin(*Cucurbita moschata*)	6	2	33.3
				Cucumber (*Cucumis sativus*)	11	5	45.5
				Bitter gourd *(Momordica charantia*)	8	3	37.5
				Bottle gourd (*Lagenaria siceraria)*	5	1	20
				Round melon (*Praecitrullus fistulosus*)	7	3	42.9
5	Plandari	33.7145° N, 73.6860° E	4500	Pumpkin (*Cucurbita moschata*)	5	2	40
				Cucumber (*Cucumis sativus*)	13	5	38.5
				Bitter gourd (*Momordica charantia*)	15	6	40
				Round melon (*Praecitrullus fistulosus*)	12	4	33.3
				Bottle gourd (*Lagenaria siceraria)*	6	1	16.7

Table 1. Continued...

Sr. No	Localities	Longitude Latitude	Altitude (ft)	Hosts	No of Sample	No. of Infested Samples	Losses (%)
6	Azad Pattan	33.7399° N, 73.6124° E	1625	Pumpkin (*Cucurbita moschata*)	5	3	60
				Cucumber (*Cucumis sativus*)	17	9	52.9
				Bitter gourd (*Momordica charantia*)	13	5	38.5
				Water melon (*Citrullus lanatus*)	5	1	20
				Round melon *Praecitrullus fistulosus*)	12	5	41.7
				Bottle gourd (*Lagenaria siceraria)*	7	2	28.6
7	Soli	74.1572° N 33.9339°E	6132	Pumpkin (*Cucurbita moschata*)	3	1	33.3
				Cucumber (*Cucumis sativus)*	9	4	40
				Bottle gourd (*Lagenaria siceraria)*	6	1	16.6
8	Khauta	33.6120° N, 73.5123° E	4541	Pumpkin (*Cucurbita moschata*)	3	1	33.3
				Cucumber (*Cucumis sativus*)	8	3	37.5
				Bottle gourd (*Lagenaria siceraria*)	4	1	25
				Round melon (*Praecitrullus fistulosus*)	6	2	33.3

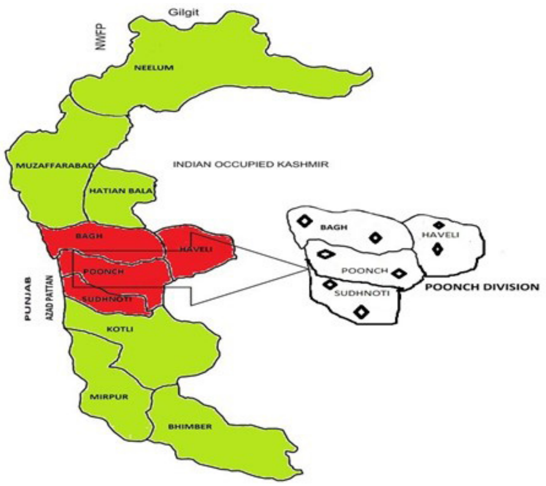

Figure 2. Map of selected localities showing distribution of *B. diversa* in study area.

(Fipronil) were used alone and in combination with each other to find out their toxicity effect. The chemical samples were obtained from the following pesticide companies viz., Syngenta, FMC and Bayer for research purposes.

2.3. Bioassays

Two bioassay methods, slice dip method for maggots and topical method for adult were used. Field recommended dose of all the insecticides were selected along with their five serial dilution and control. For slice dip method, host vegetables were cut into 3cm circular shape slice with the help of slicer cutter. Five slices were dipped in each recommended insecticide concentration along with their serial dilution and control for 10 to 15 seconds and surface dried on tissue papers. Treated slices were then placed in petri plates having filter papers beneath. Ten 3rd instar larvae were released in each petri plate at each concentration level and for control, slices were dipped in untreated distilled water. Stock solution (field recommended dose) and their different serial dilutions were considered as treatments and each treatment was replicated five times. All petri plates were covered with tight covering lids to avoid larval escape and placed under controlled laboratory conditions. Mortality as end point was recorded after 3, 6, 8 and 24 hours of application. Effectiveness of mixture insecticides were tested by mixing each insecticide with other at field recommended dose along with their serial dilution. Same procedure was repeated and mortality was observed after 3, 6, 8, and 24 hours of application. Synergistic effect was calculated when the mortality of larvae on mixture insecticides were higher than individual insecticide(s).

Efficacy of these four insecticides and their mixture were also tested on adult stage of fruit fly species by using topical application method. Adult fruit flies of 2-3 days old were used in this experiment. These collected adults were released in falcon tubes of 50ml volume with five adults in each tube. All tubes were exposed to low temperature for 20-30 seconds to slow down their flying activity. Insecticides were applied topically on thoracic region (Wang et al., 2013; Alves et al., 2024) of adult by using Hamilton micro applicator with the aid of dissecting microscope. Each fly was treated with 0.25µl of insecticide and immediately released in labeled falcon tube and capped with tight lid having small hole for ventilation. Small cotton balls were socked with sucrose solution and placed in the bottom of tube for adult diet, placed under controlled laboratory conditions and mortality counts were made after 3, 6, 8, and 24 hours holding period. Fruit flies lying in the bottom of tubes and unable to fly or climb were counted as dead. Same procedure was repeated on adult to test the efficacy of mixture insecticides and mortality data was recorded.

Statistical analysis: Mortality data was subjected to Probit analysis using Polo-PC software (Abbott, 1925; Finney, 1947).

3. Results and Discussion

Comparison of LC_{50} values of four insecticides revealed cypermethrin most effective against 3rd instar larval stage with least (7.2 (1.040±0.214)) values after 24 hours followed by imidacloprid (27.5(3.013±0.708)) chlorpyriphos (36.1(1.156±0.225)) and fipronil (83.0(1.469±0.245)) (Table 2). After 8 hours, [(37.2 (2.515±0.429)] and 6 hours [119.5 (1.123±0.200)] imidacloprid killed more insects than other insecticides. Similarly, 3 hours LC values revealed cypermethrin to be the most effective in comparison to others (Table 2). At 24 hour values of comparative ratio (CR) revealed that cypermethrin is most effective followed by imidacloprid which is 3.8 times less toxic than cypermethrin (Table 2). Similarly, chlorpyriphos is 5 times less toxic than cypermethrin followed by fiproil (11) (Table 2). Values of CR after 8 hours revealed that imidacloprid (1) is most effective followed by cypermethrin (1.6), chlorpyriphos (4.5) and fipronil (5) (Table 2). Values of CR of insecticides after 6 hours revealed that cypermethrin (1) and imidacloprid (1) are most effective followed by chlorpyriphos (2.1) and Fipronil (3.6) (Table 2). Values of CR after 3 hours revealed that cypermethrin (1) is most effective followed by imidacloprid (1.4), chlorpyriphos (3.0) and fipronil (3.6) (Table 2). For adult fruit flies, imidacloprid was the most effective with least LC_{50} [3.5 (1.886±0.437)] values after 24 hours followed by cypermethrin [17.3 (0.748±0.193)], fipronil [92.9 (1.23±60.298)] and chlorpyriphos [26.2 (0.742±0.205)], respectively (Table 3).

Similarly results showed in Table 3 revealed that Imidacloprid was most effective insecticides with maximum mortality was observed after the application of 8 hours with least LC_{50} [7.3 (1.624±0.267] and minimum mortality observed after the application of 3 hours with LC_{50} **[**80.3 (1.091±0.208)]. Values of CR for adult fruit flies revealed that imidacloprid (1) is most effective after 24, 8, 6 and 3 hours followed by cypermethrin, chlorpyriphos and fipronil (Table 3).

Maximum mortality of larval stage was observed in chlropyrifos+ cypermethrin with LC_{50} [11.3 (1.085±0.245)] after 24 hours observation. Similarly, mortality of fruit fly gradually on others insecticides combination after 24 hours observation as followed by cypermethrin+ fipronil (31.0 [1.308±0.251)], chlropyrifos+ fipronil [40.1 1.310±0.249], imidacloprid + cypermethrin [51.5 1.476±0.283], imidacloprid+ fipronil [55.5 (1.784±0.403)]. Minimum mortality at larval stage was observed at the combination of imidacloprid + chlropyrifos with LC_{50} [59.7 (1.429±0.322)]. Combination mixture of recommended field dose of chlropyrifos+ cypermethrin also proved most effective to kill maximum fruit fly larvae after the observation of 8,6 and 3 hours with least LC_{50} values [61.0 (0.931±0.196], [275.2 (1.111±0.210], [630.8 1.264±0.255] respectively (Table 4).

Overall results revealed that cypermethrin has been proved to be more effective in combination with chlorpyriphos and alone on 3rd larval instar of *B. diversa* (Table 4). Least value of CR for combination of cypermethrin +chlorpyriphos (1) after 24 hours revealed that this combination is more effective followed by cypermethrin+ fipronil (1.47), chlorpyriphos+ fipronil (3.6), imidacloprid+cypermethrin (4.6) and imidacloprid+fipronil (5) respectively (Table 4). After 8 hours CR values showed that cypermethrin + chlorpyriphos (1) is most toxic followed by imidacloprid+ fipronil (2.6), cypermethrin+ fipronil (2.8), chlorpyriphos+fipronil (2.9), imidacloprid+chlorpyriphos (3) and imidacloprid+fipronil (3.6) respectively (Table 4). After 3 and 6 hours least value CR value of cypermethrin + chlorpyriphos (1) and maximum value of imidacloprid+ chlorpyriphos (5.7, 6.8) shown that former is most and later is least toxic to larval instar (Table 4).

Combination results of these insecticides on adult stage revealed that chlropyrifos+ cypermethrin was most effective against adult stage of *B. diversa* with least LC_{50} [2.5 (0.759±0.252)] after 24hour observation followed by cypermethrin+ fipronil [5.3 (0.880±0.280)], imidacloprid+ cypermethrin [18.8 (1.267±0.346)], chlropyrifos+ fipronil [27.1 (1.305±0.275)], imidacloprid+ fipronil [54.0 (2.532±0.764)] and imidacloprid+ chlropyrifos [176.1 (1.666±0.258). Similarly, mortality after the application of chlropyrifos+ cypermethrin with least LC_{50} values [24.9 (1.097±0.213), 62.7 (1.181±0.203), 241.9 (1.352±0.218)] after 8, 6 and 3 hours respectively shown that this combination as most effective (Table 5). After 24, 8, 6 and 3 hours least CR value of chlorpyriphos + cypermethrin (1) shown that this combination of insecticide is most toxic to adult fruit flies as compared to other applied (Table 5). At 24 hours CR value of cypermethrin+ fipronil (2.1) which is two times less toxic than chlorpyriphos + cypermethrin (1) and toxicity of other combined

Table 2. Toxicity of four different insecticides on 3rd larval instar of fruit fly (*Bactrocera diversa*) under laboratory conditions using topical application and slice dip bioassay method.

Insecticides	Time of exposure	LC50 (FL at 95%)	LC90 (FL at 95%)	Slope ± SE	chi-square	Comparative Ratio
Imidacloprid	3hr	276.1 (19.2-400.3)	1,546.5 (460.8-12, 234.2)	1.713±0.230	4.0	1.4
	6hr	119.5(91.67- 302.5)	1,653.1 (506.65- 5,123.6)	1.123±0.200	3.4	1
	8hr	37.2 (18.567- 120.564)	120.3(95.547-3,990.745)	2.515±0.429	1.6	1
	24hr	27.5 (8.56-102.35)	73.2 (33.057-129.818)	3.013±0.708	1.5	3.8
Chlorpyriphos	3hr	576.7 (394.027-1109.142)	5,416.9 (2,254.9-31,217)	1.317±0.231	2.8	3.0
	6hr	260.7 (192.706-386.127)	2,669.9(1311.9-10102)	1.268±0.207	2.8	2.1
	8hr	171.0 (123.773-240.205)	1,994.6 (1007.3-7,321.2)	1.201±0.202	1.0	4.5
	24hr	36.1 (15.131-56.906)	463.6 (286.783-1,180.616)	1.156±0.225	0.4	5
Cypermethrin	3hr	191.2 (92.547-3,921.745)	1,464.8(447.13-4,636.0)	1.449±0.272	3.9	1
	6hr	127.2 (83.234- 274.037)	1,427.0 (531.07-11,191)	1.221±0.225	2.5	1
	8hr	62.8 (40.382-134.670)	1,983.1(530.85-58,918)	0.855±0.196	0.5	1.6
	24hr	7.2 (2.648-11.742)	122.1(69.879-393.232)	1.040±0.214	1.1	1
Fipronil	3hr	698.8 (539.439-990.050)	4,210.0 (2,422.5-10,815)	1.643±0.233	2.3	3.6
	6hr	470.4 (294.929-901.386)	2,854.9 (1,296.2-24,961)	1.636±0.219	4.1	4
	8hr	207.2 (103.393-337.500)	1,410.2 (711.469-9,219.831)	1.539±0.216	4.0	5.5
	24hr	83.0 (46.057-117.818)	617.5 (429.363-1,139.870)	1.469±0.245	2.3	11

FL: fiducial limit.

Table 3. Toxicity of four different insecticides to adult fruit fly (*Bacterocera diversa*) under laboratory conditions using topical application and slice dip bioassay method.

Insecticides	Time of exposure	LC50 (FL at 95%)	LC90 (FL at 95%)	Slope ± SE	chi-square	Comparative Ratio
Imidacloprid	3hr	80.4(54.791-152.514)	1,201.3(442.82-10157)	1.091±0.208	2.4	1
	6hr	15.5 (7.957-23.358)	363.2 (160.727-2,352.853)	0.936±0.198	0.8	1
	8hr	7.3 (4.105-10.360)	45.0(32.780-74.829)	1.624±0.267	1.4	1
	24hr	3.5 (1.041-5.877)	16.9 (12.337-25.581)	1.886±0.437	1.1	1
Chlorpyriphos	3hr	1,667.2 (779.80-12,156)	31,458.2(5,995.0-32,540)	1.005±0.246	1.2	20
	6hr	1,176.1(611.65-5,612.6)	22,878.0(5,029.1-31,990)	0.994±0.230	0.4	75.8
	8hr	628.6 (345.470-2,758.518)	28,074.1(4,867.2-52,728)	0.777±0.200	0.9	86.1
	24hr	26.2 (3.235-54.078)	1,400.2 (621.44-10,527.2)	0.742±0.205	0.7	7.4
Cypermethrin	3hr	422.0 (178.89-4,752.5)	8,806.2 (1,417.6-21,156)	0.971±0.250	0.3	5.2
	6hr	211.4(105.044-1,318.045)	7,101.9(1,194.1-12,868)	0.840±0.212	0.3	13.6
	8hr	104.4(53.839-787.209)	11,899.0 (1,220.8-12,094)	0.623±0.194	0.9	14.3
	24hr	17.4 (7.299-28.356)	898.9 (2,67.54-31,367)	0.748±0.193	1.0	4.9
Fipronil	3hr	1,651.9 (951.95-5447.2)	30,612.1 (7961.0-82,065	1.011±0.220	0.7	20.5
	6hr	437.8 (288.625-759.467)	12,043.0(3,855.4-19,232)	0.890±0.195	2.0	28.2
	8hr	92.9 (39.561-145.689)	1,676.1(900.67-6,342.3)	1.020±0.209	0.1	12.7
	24hr	24.9(3.681-52.143)	271.5 (180.926-497.262)	1.23±60.298	2.0	7.1

insecticides decreased as imidacloprid + cypermethrin (7.6), chlorpyriphos + fipronil (10.8), imidacloprid + fipronil (21.6), imidacloprid+ chlorpyriphos (70.4) respectively (Table 5). After 8 hours chlorpyriphos+ fipronil (4.2) is 4 times less toxic than chlorpyriphos + cypermethrin (1) and toxicity decreases for other combinations (Table 5).

Table 4. Toxicity of four different insecticides in combination to 3rd larval instars of fruit fly (*Bactrocera diversa*) under laboratory conditions using topical application and slice dip bioassay method.

Insecticides	Time of exposure	LC50 (FL at 95%)	LC90 (FL at 95%)	Slope ± SE	chi-square	Comparative Ratio
Imidacloprid+ Chlorpyriphos	3hr	3,611.1(2,018.9- 13,155.)	70,885.1 (17353- 2,34,657)	0.991±0.219	0.3	5.7
	6hr	1,881.4 (1092.0-6,513.4)	8,9214.1(1,6547-1,35,248)	0.765±0.197	1.0	6.8
	8hr	220.6 (114.434-367.975)	3,321.4 (1,487.5- 11,908)	1.088±0.207	4.0	3.6
	24hr	59.7 (13.473-111.680)	471.2 (326.665- 782.576)	1.429±0.322	0.8	5.4
Imidacloprid+ Cypermethrin	3hr	1767.5 (1036.4-5419.3)	28,103.3 (5419.3-55,636)	1.067±0.224	2.0	2.7
	6hr	685.1 (471.596-1,228.899)	11422.2 (4,229.9-96,591)	1.049±0.202	2.0	2.4
	8hr	188.4 (114.434-267.975)	2,946.9 (1,487.5-11,908)	1.073±0.201	1.5	3
	24hr	51.5 (13.473-111-680)	380.0 (326.665-782.576)	1.476±0.283	0.5	4.6
Imidacloprid+ Fipronil	3hr	1,168.3 (896.587-1,687.752)	6,831.3 (3,886.5-18,029)	1.671±0.240	1.9	1.8
	6hr	382.3 (279.692-507.404)	3,367.5 (1,985.1-8,504.4)	1.356±0.207	1.1	1.3
	8hr	161.8 (112.661-208.884)	779.0 (581.047-1,223.128)	1.878±0.267	0.6	2.6
	24hr	55.5 (16.987- 91.967)	290.1(212.013-448.563)	1.784±0.403	2.0	5
Chlorpyriphos+ Cypermethrin	3hr	630.8(371.938-1,880.755)	6,510.9 (2,096.7-84,297)	1.264±0.255	0.3	1
	6hr	275.2 (184.855-546.482)	3914.0 (1,428.2-33,176)	1.111±0.210	0.7	1
	8hr	61.0 (35.041-90.730)	1,450.4 (601.39-1,0951	0.931±0.196	1.0	1
	24hr	11.3 (2.482-21.548)	171.3 (108.828-423.774)	1.085±0.245	1.4	1
Chlorpyriphos+ Fipronil	3hr	1,054.8 (721.580- 2,038.247)	7,539.3 (3,345.7-37,258)	1.500±0.258	1.8	1.7
	6hr	645.0 (456.176- 1,128.646)	6,834.4 (2,931.7-36,171)	1.250±0.216	2.9	2.3
	8hr	172.8 (117.813- 237.470)	2,089.5 (1,120.2- 6,810.3)	1.184±0.201	2.2	2.9
	24hr	40.1 (15.861-64.758)	381.6 (260.748- 737.212)	1.310±0.249	1.8	3.6
Cypermethrin+ Fipronil	3hr	1,072.0 (596.387- 3,974.181)	1,8874.1 (4,735.9-57,919)	1.029±0.227	1.3	1.6
	6hr	453.0 (297.459-961.297)	9,061.1 (12,936-28,587)	0.985±0.203	2.6	1.6
	8hr	171.5 (86.339 -374.049)	3,523.7 (821.207-4,995.344)	0.976±0.196	1.2	2.8
	24hr	31.0 (1.907- 63.430)	295.9 (159.620-2,247.388)	1.308±0.251	3.5	1.47

Similarly, after 3 and 6 hours toxicity of insecticides combination was recorded less than chlorpyriphos + cypermethrin (1) (Table 5).

Comparison of LC_{90} values revealed that imidacloprid is most effective with least value [73.106 (3.013±0.708)] after 24 hours followed by cypermethrin [122.1 (1.040±0.214)], chlorpyrifos [463.6 (1.156±0.225)] and fipronil [1410.2 (1.539±0.216)] on 3rd larval instar of *Bacterocera diversa* (Table 2). Bioassay results shown that Imidacloprid is also effective after 8 hours with least LC_{90} values (120.3 (2.515±0.429)) whereas cypermethrin was affective after 6 and 3 hours with least LC_{90} values (1427.0 (1.221±0.225), 1464.8 (1.449±0.272)) respectively (Table 2). Mortality of 90% of adult population of *Bacterocera diversa* was observed after the application of imidacloprid with least value of LC_{90} [16.9 (1.886±0.437)] after 24 hours followed by fipronil [271.5 (1.23±60.298)], cypermethrin [898.9 (0.748±0.193)] and chlorpyrifos [1400.2 (0.742±0.205)]. Similarly results shown that imidacloprid is most effective after 8, 6 and 3 hours with least LC_{90} values [45.0 (1.624±0.267), 363.2 (0.936±0.198), 1201.3 (1.091±0.208)] respectively (Table 3).

Results shown that combination of cypermethrin+chlorpyrifos is most effective against 3rd larval instar of *B. diversa* with least value of LC_{90} [171.3 (1.085±0.245)] after 24 hours followed by Imidacloprid+ Fipronil [290.1 (1.784±0.403)], cypermethrin+ fipronil [295.9 (1.308±0.251)], imidacloprid+ cypermethrin [380.0 (1.476±0.283)], chlropyrifos+ fipronil [381.6 (1.310±0.249)] and imidacloprid+ chlropyrifos

Table 5. Toxicity of four different insecticides in combination to adults of fruit fly (*Bacterocera diversa*) under laboratory conditions using topical application and slice dip bioassay method.

Insecticides	Time of exposure	LC50 (FL at 95%)	LC90 (FL at 95%)	Slope ± SE	chi-square	Comparative Ratio
Imidacloprid+ Chlorpyriphos	3hr	31,01.2 (1,863.9-8,707.7)	47,408.1(14,081-76,634)	1.082±0.221	0.8	12.8
	6hr	1,108.2 (769.067-1,883.51)	19,294.1(7,298.3-25,790)	1.033±0.200	1.2	17.6
	8hr	596.4(412.003-853.460)	8,851.2 (4,150.9-40,827)	1.094±0.199	0.6	23.9
	24hr	176.1(108.651-239.901)	1,035.1 (752.466-1,716.185)	1.666±0.258	2.8	70.4
Imidacloprid+ Cypermethrin	3hr	1,535.7(975.536-3,625.311)	17,332.1(6,120.1-26,101)	1.218±0.232	0.1	6.3
	6hr	796.5 (552.155-1,432.357)	10,841.1(4,263.4-7,4151)	1.130±0.207	1.3	12.7
	8hr	139.4 (63.286-215.985)	20,14.8 (1,005.2-10,441)	1.105±0.206	3.1	5.5
	24hr	19.0 (1.255-45.246)	194.0 (117.727-331.108)	1.267±0.346	2.3	7.6
Imidacloprid+ Fipronil	3hr	926.0 (701.814- 1,342.248)	7,017.7 (3,773.3-21,022)	1.457±0.218	0.7	29
	6hr	352.2 (266.235-451.151)	2,301.1 (1,517.488-4,511.409)	1.572±0.214	1.3	5.6
	8hr	113.4 (64.880-159.242)	687.8(500.803- 1,139.066)	1.637±0.266	1.7	4.5
	24hr	54.0 (10.135-86.797)	173.3 (125.002-252.833)	2.532±0.764	1.3	21.6
Chlorpyriphos+ Cypermethrin	3hr	241.9 (175.2-393.4)	2,144.9 (1,015.8-8,652.8)	1.352±0.218	1.8	1
	6hr	62.7 (41.9- 86.3)	763.8 (413.3-2,459.9)	1.181±0.203	0.6	1
	8hr	24.9 (2.296- 49.549)	366.9 (165.525-8,367.205)	1.097±0.213	3.4	1
	24hr	2.5 (0.005-10.193)	121.9 (65.688-500.367)	0.759±0.252	0.8	1
Chlorpyriphos+ Fipronil	3hr	657.5 (427.452-1,500.658)	3,819.2 (1,620.9-4,712.5)	1.677±0.246	3.1	2.7
	6hr	256.7 (180.204-374.459)	3,816.5 (1,736.4-18,816)	1.093±0.199	1.2	4
	8hr	107.0 (45.450-169.957)	3,792.4 (1,417.4-50,012.2)	0.827±0.197	2.0	4.2
	24hr	27.1 (7.442-48.764)	260.0 (179.261-474.240	1.305±0.275	1.5	10.8
Cypermethrin+ Fipronil	3hr	735.0 (496.496-1,464.847)	6,147.3 (2,579.2-34,948)	1.389±0.244	0.7	3
	6hr	435.1(260.865-1,278.818)	20,092.1 (4021.4-260.865)	0.770±0.196	0.4	6.9
	8hr	114.8 (52.337-190.844)	6,733.4 (1,778.1-39,886)	0.725±0.192	0.5	4.6
	24hr	5.4 (0.165-16.728)	154.0 (94.539- 303.974)	0.880±0.280	2.2	2.1

[471.2 (1.429±0.322)] respectively (Table 4). After 8 and 6 hours imidacloprid+ fipronil with least LC_{90} values [779.0 (1.878±0.267), 3367.5 (1.356±0.207], and after 3 hours cypermethrin + chlorpyrifos with least LC_{90} values [6510.9 (1.264±0.255)] respectively were proved as most effective combination (Table 3).

Combination of cypermethrin+chlorpyrifos also killed 90% of adult population of *B. diversa* with least value of LC_{90} [121.9 (0.759±0.252)] after 24 hours of exposure followed by cypermethrin+ fipronil [154.0 (0.880±0.280)], imidacloprid+fipronil [173.3 2.532±0.764), imidacloprid+ cypermethrin [194.0 (1.267±0.346)], chlropyrifos+ fipronil [260.0 (1.305±0.275)] and imidacloprid+ chlropyriphos [1035.1 (1.666±0.258)] respectively (Table 5). cypermethrin+chlorpyrifos with least LC_{90} values [366.9 (1.097±0.213, 763.8 (1.181±0.203), 2144.9 (1.352±0.218)] after 8, 6 and 3 hours respectively shown that that this combination is most effective on all time interval and toxicity increase with increase in time (Table 5).

Overall results showed that cypermethrin was the most effective to kill 50% of both larval and adult stage followed by imidacloprid. Imidacloprid most effectively killed 90% of both larval and adult population after 24 hours. Cypermethrin in combination with chlorpyrifos most effectively killed 50 and 90 percent of both larval and adult stage of *B. diversa* after 24 hour of exposure. Toxicity of each insecticide increased with exposure for longer time and increased dose. cypermethrin caused 90% mortality of *B. cucurbitae* (Rana et al., 2015). Cypermethrin also shown good efficacy against another tephritid fruit fly *B. tyroni*.

Toxicity assessment of various insecticides on *Bactrocera invadens* species also proved the efficacy of cypermethrin and chlorpyriphos (Abdullahi et al., 2020). Toxicity of cypermethrin increased with increased dose on *Bactrocera* species (Lin et al., 2013) is in line with results of this study. Efficacy of different insecticides was tested on three populations of *Bacterocera minax* which proved highest toxicity of Chlorpyriphos (Liu et al., 2015) while in our study chlorpyrifos in combination with cypermethrin is also effective against larval and adult stage of *B. diversa*. Imidacloprid was proven least effective against *B. zonata* as compared to trichlorfon, λ-cyhalothrin (Khan and Naveed, 2017). Imidacloprid has been reported as least effective while (Yee and Alston, 2006) reported effective results of imidacloprid against tephritid fruit fly while in our study imidacloprid is effective after cypermethrin. Other than *Bactrocera* species imidacloprid killed more *Anastrepha suspensa* at 8% active Ingredient after 2-72 hours exposure (Liburd et al., 2004).

B. dorsalis is more susceptible to fipronil than *B. cucurbitae* by oral and topical route of exposure (Stark et al., 2009). Fipronil is most toxic against two populations of *B. dorsalis* from two different provinces of China whereas cypermethrin and imidacloprid are least toxic respectively (Wang et al., 2013). Insecticide application for the management of fruit flies should be taken as a tool in combination with other ecofriendly techniques.

Tephritid fruit flies are one of the major pest insect of wide range of fleshy fruits and vegetables among which *Bacterocera diversa* is a serious pest of Cucurbitaceae and distributed from Pakistan to Vietnam (Vargas et al., 2015). *B. diversa* has been reported from various parts of Asian region including Indo Pak (David and Ramani, 2011) whereas Prabhakar et al. (2012) reported *B. diversa* from cucurbit fields of Himachal Pradesh, India. Along with many other pests of cucurbits fruit flies alone cause 72-80% loss (Sood et al., 2010). As a pest of cucurbits *B. diversa* cause losses by oviposition in flowers (Vargas et al., 2015; Molla et al., 2000), decrease yield of cucurbits by dropping of infested flowers. It causes serious damage to Pumpkin, bottle gourd, ash gourd, ivy gourd, sweet gourd, ribbed gourd, sponge gourd, cucumber, watermelon, snake gourd, bitter (Syed, 1970; Drew and Romig, 2013). In IndoPak region including Bangladesh with rise in temperature and flowering of cucurbits this pest become active and causes losses (Molla et al., 2000; Syed, 1970). Kabir et al. (1991) reported 42% losses of cucurbits in Bangladesh who is major exporter of bottle gourd, ash gourd and ribbed gourd to Middle East (Naqvi, 2005).

During the present study maggots of *B.diversa* are found actively feeding inside the cucurbit host (Table 1) collected from different localities of Poonch division of Azad Jammu & Kashmir, Pakistan (Figures 1-2) whereas Royer et al. (2018) reported that *B. diversa* only infest flowers not fruits (Syed, 1970; Kabir et al., 1991). *Bactrocera diversa* was collected from infested pumpkin, cucumber, bitter gourd, watermelon, round melon, bottle gourd brought to laboratory and then emerged in cages. This species is distributed from lower altitude to high altitudes ranging from 1625- 6132 ft from sea level where cucurbits are growing in study area. *Bactrocera diversa* was found to cause 40- 60% losses in pumpkin (*Cucurbita moschata*), 37.5-52.9% to cucumber (*Cucumis sativus)*, 37.5- 45% in bitter gourd (*Momordica charantia)* 16.6-37.5% in bottle gourd (*Lagenaria siceraria)* at study site. Losses caused by Tephritid fruit flies to cucrbits in Himachel perdesh and Himalayas of India ranging from 70 -80% are reported by (Gupta and Bhalla, 1990; Sood et al., 2010). Badii et al. (2015) inferred that cucurbits are primarily attacked by 3 species of *Dacus* and *Bactrocera cucurbitae.* It was observed that losses were higher at lower altitudes as compared to higher elevation. Localities like Bagh Azad Patan Plandari and Hajira have subtropical type of weather conditions whereas Rawalakot, Kahuta, Soli (Jalalabad) have temperate type of climatic conditions where winters are very harsh and summers are mild (Nazir et al., 2014). Nine species of the Tephritid fruit flies from Poonch division of Azad Jammu & Kashmir were recorded (Zubair et al., 2019) but *Bactrocera diversa* was not reported from region of Azad Jammu & Kashmir. Methyl eugenol is a weak attractant for *B. diversa* and very few specimens or none can be collected by using this attractant in the trap (Royer et al., 2018) During the present study a large number of *B. diversa* was collected from infested cucurbits in the laboratory for the first time.

In conclusion, our study suggests cypermethrin be used to control *B. diversa* followed by imidacloprid and in combination with chlorpyriphos, however field trials are necessary to validate our findings.

4. Conclusion

Findings of this study provide a baseline for the management of this invasive species of *Bactrocera diversa* in cucurbit crops and in this area. Timely application of cypermethrin in combination with chlorpyriphos is an effective control option for the farmers of this area who may prevent their cucurbit losses by this pest.

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